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Drinking Water Criteria Document for 1,2-Dibromo-3-Chloropropane (DBCP) (Final Draft)

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DRINKING WATER CRITERIA DOCUMENT FOR 1,2-DIBROMO-3-CHLOROPROPANE (DBCP)

Prepared for

OFFICE OF DRINKING WATER

Prepared by

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The Office of Drinking Water (ODW), U.S. Environmental Protection Agency has prepared a Drinking Water Criteria Document on dibromochloropropane. This Criteria Document is an extensive review of the following topics:

- Physical and chemical properties of dibromochloropropane
- Toxicokinetics and human exposure to dibromochloropropane
- Health Effects of dibromochloropropane in humans and animals
- Mechanisms of toxicity of dibromochloropropane
- Quantification of toxicological effects of dibromochloropropane

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LIST OF ABBREVIATIONS

AADI	Adjusted acceptable daily intake
ADI	Acceptable daily intake
BUN	Blood urea nitrogen
CNS	Central nervous system
DNA	Deoxyribonucleic acid
FSH	Follicle stimulating hormone
GI	Gastrointestinal
GOT	Glutamic oxaloacetic transaminase
GPT	Glutamic pyruvic transaminase
GSH	Glutathione
LC_{50}	Concentration lethal to 50% of recipients
LD_{50}	Dose lethal to 50% of recipients
LDH	Lactic dehydrogenase
LH	Luteinizing hormone
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
OCT	Ornithine carbamyl transferase
RNA	Ribonucleic acid
TWA	Time-weighted average
UDS	Unscheduled DNA synthesis

I. SUMMARY

1,2-Dibromo-3-chloropropane (DBCP) is a colorless liquid when pure and is used as a nematocidal fumigant. An EXAMS model predicted volatilization as the dominant removal process from water (half-life = 4-24 days), hydrolysis, oxidation and sorption being much less important. Biodegradation may be important with a half-life of ~2 weeks; however, insufficient data are available to make a good prediction.

DBCP is absorbed by the GI tract, the lungs and the skin of animals as inferred from data on its systemic toxicity following administration by these routes. DBCP is widely distributed throughout the body where reactive metabolites bind to tissue macromolecules. Highest levels of unbound parent DBCP are found in adipose tissue where it persists for at least 24 hours. DBCP can cross the placenta of pregnant rats. Urinary metabolites found in rats were mercapturic acid conjugates, β-chlorolactic acid, β-bromolactic acid and 2-bromoacrylic acid. Proposed metabolic pathways involve intermediates including epihalohydrins and other reactive epoxides and 2-bromoacrolein, a potent mutagen. Information on elimination is sparse. Elimination of parent DBCP by the lungs of rats amounted to ~14% in 4 hours. No parent DBCP was detected in urine.

The acute oral LD_{50} of DBCP ranged from 170-350 mg/kg bw in rats, 260-410 mg/kg bw in mice, 210-316 mg/kg bw in guinea pigs and 180-440 mg/kg bw in rabbits. Effects of 1-14 days of administration of DBCP in rats included reduced body weight, impaired renal function, histopathologic damage to renal tubules, hepatocellular necrosis, testicular and epididymal atrophy, necrotic germ cells in testis, loss of spermatogenic elements in the testis and necrotic lesions in the lungs. The estimated acute oral NOAEL in adult rats was 20 mg/kg bw, and the acute LOAEL with subcutaneous injection was 20 mg/kg bw in 6-day-old rats.

Similar effects were observed for subchronic oral exposure. A NOAEL for subchronic (90 days) dietary administration of DBCP was 5 mg/kg diet (0.5 mg/kg bw/day) and for 8 months of gavage administration was 0.05 mg/kg bw/day. Increased glucose and protein levels in urine were evident in male rats given DBCP in drinking water for 5 days. Mice and rats treated chronically by gavage with DBCP had high rates of mortality and high incidences of toxic tubular nephropathy. Effects of acute and subchronic inhalation exposures of rats to DBCP also included damage to kidneys, testes, liver and lungs. In addition, histopathologic lesions in the nasal cavity of rats and mice occurred with concentration-related increased incidences. Chronic inhalation exposure in rats and mice resulted in high mortality, toxic tubular nephropathy and lesions of the nasal cavity and stomach. Following chronic oral administration of DBCP, rats and mice had highly significant increased incidences of squamous cell carcinoma of the forestomach. Adenocarcinomas of the mammary glands were significantly increased in treated female rats and carcinomas of the renal tubules were induced in rats of both sexes. Chronic inhalation exposure to DBCP resulted in induction of nasal cavity tumors in rats and mice. DBCP was positive as a tumor initiator on the skin of mice but negative as a whole carcinogen on skin; however, distant tumors of lung and stomach were found after repeated dermal application.

DBCP was mutagenic in prokaryotic and eukaryotic systems, resulting in reverse mutations in _Salmonella typhimurium_ with and without metabolic activation, recessive lethal mutations in _Drosophila melanogaster_, dominant lethal mutations in rats and chromosomal damage in rats and cultured Chinese hamster cells. Some of the mutagenic activity of DBCP has been attributed to epichlorohydrin, a metabolite and a contaminant of DBCP; however, DBCP free of epichlorohydrin was also shown to have mutagenic activity with metabolic activation. No teratogenic effects of DBCP were found in rats or rabbits. DBCP was fetotoxic at doses toxic to dams. Male rabbits treated at high doses became infertile; the infertility was reversible.

The threshold for taste and smell of DBCP in water was reported to be 0.01 mg/ℓ. Epidemiological studies of chemical company workers and agricultural workers have indicated that occupational exposure to DBCP results in reduced spermatogenesis. The incidences of oligospermia and azoospermia were related to intensity and duration of exposure. Recovery from oligospermia and azospermia depended upon intensity and duration of exposure and the length of time since the last DBCP exposure. Occupational exposure to DBCP has also been associated with nondisjunction of the y-chromosome and with increased incidences of spontaneous abortion in women whose husbands had been exposed.

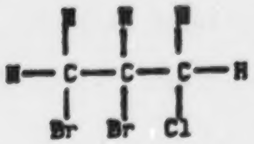
The toxic action of DBCP has been attributed to the formation of reactive metabolites that act as alkylating agents, binding to nucleophilic sites on biological macromolecules. Among the possible reactive compounds are epoxides and a bromonium ion. DBCP and its intermediate metabolites, epichlorohydrin and α-chlorohydrin, may produce toxic effects in the testis, epididymis and forestomach by similar mechanisms. The renal effects of DBCP were unlike those produced by oxalic acid, epichlorohydrin or α-chlorohydrin.

Quantitative risk assessment for DBCP was based on animal toxicity data following oral administration. Toxicity in humans was observed after occupational exposure to concentrations of <9.7 mg/m^3, but extrapolation from the inhalation route to the oral route is less desirable when usable studies using oral dosing are available, which is the case for DBCP. A 1-day HA for DBCP for a 10 kg child is 0.2 mg/ℓ (0.2 mg/day or 0.02 mg/kg bw) and for a 70 kg adult is 0.7 mg/ℓ (1.4 mg/day or 0.02 mg/kg bw). A 10-day HA for a 10 kg child is 0.05 mg/ℓ (0.05 mg/day or 0.0050 mg/kg bw/day) and for a 70 kg adult is 0.175 mg/ℓ (0.35 mg/day or 0.0050 mg/kg bw/day). These HA levels are based on noncarcinogenic effects and are above the 0.01 mg/ℓ level reported as the threshold for taste and smell. The carcinogenicity of DBCP in the chronic treatment studies in animals precludes calculation of a lifetime AADI based on noncarcinogenic effects. The U.S. EPA's Carcinogen Assessment Group has used the multistage model and carcinogenicity data from a lifetime feeding study with DBCP in rats to calculate a 1×10^{-6} upper limit of risk from lifetime exposure to 0.025 μg DBCP/ℓ of water, assuming a daily intake of 2 ℓ of drinking water.

II. GENERAL INFORMATION AND PROPERTIES

Structure and Identification

1,2-Dibromo-3-chloropropane (DBCP) is available under the synonyms Nemafume, Fumazone and Nemagon. It has the following structure:



$$\begin{array}{ccccccc} & & H & & H & & H & \\ & & | & & | & & | & \\ H & - & C & - & C & - & C & - & H \\ & & | & & | & & | & \\ & & Br & & Br & & Cl & \end{array}$$

The molecular formula is $C_3H_5Br_2Cl$. The molecular mass is 236.36.

The Chemical Abstract Service (CAS) registry number for this compound is 96-12-8, and the Registry of Toxic Effects of Chemical Substances (RTECS) number is TX87500.

Physical and Chemical Properties

The physical properties of DBCP are summarized in Table II-1. The commercial product is a light yellow to brown liquid with a pungent odor, but it is colorless and clear when pure.

Stability in Water

An EXAMS (Exposure Analysis Modeling System) model (Burns et al., 1981) of the fate of DBCP in water and other data in the literature predict that biodegradation and volatilization are probably the dominant removal processes for DBCP in water.

An EXAMS model based on the data presented on Table II-1 and the hydrolysis data of Burlinson et al. (1982) ($K_H O_2 = 0.33 \times 10^{-6}$ hr^{-1}, $K_{OH} = 20.6$ M^{-1} hr^{-1}) predicted a volatilization half-life of 4.2-24

TABLE II-1

Physical Properties of DBCP

Property	Value	Reference
Boiling point	196°C	BCPC, 1977
Specific gravity (at 20°C)	2.08	Versucheren, 1983
Vapor pressure (at 21°C)	0.8 Torr	BCPC, 1977
Log octanol/water partition coefficient	2.43*	
H_2O solubility	1230 mg/ℓ (ppm)	Chiou et al., 1979
Lipid solubility	Miscible in oils	Windholz, 1983
Conversion factor	1 ppm = 9.67 mg/m³ 1 mg/m³ = 0.103 ppm	
Adsorptive capacity of activated carbon	53 mg/g	Ramanathan, 1984

*Back calculated from log soil organic matter/water partition coefficient (2.11, Hansch and Leo, 1981) by the method described in Mabey et al., 1981.

days for DBCP depending on the environment used with the model (e.g., pond, eutrophic lake, oligotrophic lake, river). The hydrolysis half-life calculated by the model was 6.0, 5.6, 39 and 44 years for a pond, eutrophic lake, oligotrophic lake and river, respectively; Burlinson et al. (1982) calculated a half-life of 141 years at 15°C and 38 years at 20°C. Based on data available for other halogenated alkanes (Mabey et al., 1981) oxidation is not predicted to be an important process. The EXAMS model also predicted that the vast majority of the compound would remain in the water column instead of adsorbing to sediments or bioconcentrating in biota.

Castro and Belser (1968) reported that the DBCP both degraded and volatilized in soil suspensions. Several soils were screened for activity; the one for which data was reported had a half-life of >3 weeks. Volatilization appeared to be more rapid than degradation in this system. n-Propyl alcohol was the principal product, probably going through allyl chloride and allyl alcohol intermediates (Castro and Belser, 1968). Given the paucity of data, it is difficult to predict the importance of biodegradation in the environment.

Summary

Dibromochloropropane (DBCP) is a colorless, clear liquid when pure, has a molecular weight of 236.36, a vapor pressure of 0.8 torr, a water solubility of 1230 mg/ℓ, and a calculated log octanol/water partition coefficient of 2.43 (BCP, 1977; Verschueren, 1983). An EXAMS model (Burns et al., 1981) predicted that volatilization would be the dominant removal process for DBCP in aquatic systems with a half-life between 4 and 24 days; whereas hydrolysis (Burlinson et al., 1982) and oxidation (Mabey et al., 1981) will be slow. Adsorption of DBCP to sediments and bioconcentrating in biota, as predicted by the EXAMS model, is low. The importance of biodegradation to the overall removal of DBCP from aquatic systems is not clear based on available data.

III. TOXICOKINETICS

Absorption

Quantitative information pertaining to the absorption of DBCP from the GI tract, by the lungs or by the skin of laboratory animals, was not located in the available literature. That DBCP can be absorbed by these routes can be inferred from the systemic toxicity that results from exposure of animals to DBCP by these routes (see Chapter V). Evidence that DBCP can be absorbed from the GI tract can be obtained from data on the distribution of the pesticide in the rat after oral exposure (Ruddick and Newsome, 1979; Kato et al., 1980) (see Distribution and Retention Section).

Distribution and Retention

Lipscomb et al. (1977) reported in a meeting abstract that maximum levels of radioactivity from [1,3-^{14}C]-DBCP were detected in most tissues (unspecified) of rats at 1-3 hours after intraperitoneal injection. The dose administered was not reported. Highest levels were found in the intestine, liver and kidneys. In the liver and kidneys radioactivity was observed to be bound to RNA, protein and DNA.

Ruddick and Newsome (1979) studied the distribution of DBCP in 25 pregnant Wistar rats and 5 vehicle controls that had been administered a dose of 25 mg DBCP/kg bw/day in corn oil by gavage on days 6-15. On day 15, 1 control and 5 treated rats were killed at each of 1, 3, 6, 12 or 24 hours after the last dose. DBCP was detected by gas chromatography. The results are presented in Table III-1. Gas chromatographic analysis of tissue extracts from control animals showed apparent DBCP levels of 3 ppb in heart, 2 ppb in kidneys and 21 ppb in fat; other tissue extracts had nondectable levels.

TABLE III-1

Amount of 1,2-Dibromo-3-chloropropane (ppm) in Tissues at Specified Times After Last of 10 Consecutive Daily Doses (25 mg/kg) to Pregnant Rats*

Sample	1 Hour	3 Hours	6 Hours	12 Hours	24 Hours
Fetus	0.008 ± 0.002	0.030 ± 0.015	0.030 ± 0.013	0	NA
Spleen	0.011 ± 0.001	0.027 ± 0.009	0.023 ± 0.008	0	NA
Brain	0.101 ± 0.028	0.164 ± 0.048	0.211 ± 0.081	0.008 ± 0.001	NA
Heart	0.123 ± 0.027	0.249 ± 0.069	0.260 ± 0.116	0.013 ± 0.004	NA
Lung	0.088 ± 0.014	1.12 ± 0.602	0.646 ± 0.351	0	NA
Kidney	0.016 ± 0.003	0.041 ± 0.014	0.047 ± 0.009	0	NA
Liver	0.009 ± 0.002	0.027 ± 0.012	0.021 ± 0.007	0	NA
Adipose tissue	2.53 ± 0.381	3.87 ± 0.480	5.38 ± 0.410	1.37 ± 0.275	0.170 ± 0.071
Blood	0.082 ± 0.022	0.104 ± 0.030	0.100 ± 0.045	0.011 ± 0.001	0

*Source: Ruddick and Newsome, 1979

Values are mean ± standard error of 5 samples

NA = Not analyzed

Apparent DBCP in control tissue was considered to be due to the sensitivity of the methods and not DBCP itself. Twice the levels found in the control tissues were set as the limit of detection. Peak levels in all tissues examined occurred at 3-6 hours after the last dose. The levels in most tissues declined to below detection levels at 12 hours. Highest levels of DBCP were found in abdominal adipose tissue, which accumulated the pesticide for up to 6 hours after the last dose. The level in adipose tissue declined appreciably over the next 6 hours, but relatively high levels were still detected at 24 hours. After adipose tissue, the tissues that achieved the highest concentrations of DBCP were, in descending order, lung, heart and brain. Low levels were detected in the fetus, indicating transplacental transfer.

The distribution and binding to macromolecules of radioactivity and DBCP from [3-^{14}C]-DBCP (94% radiochemical purity), administered orally in olive oil to 9-week-old male Wistar rats at doses of 20, 50, 100, 200 or 400 mg/kg bw, was studied by Kato et al. (1980). Whole-body autoradiography, at 6 hours following administration of 20 or 200 mg ^{14}C-DBCP/kg bw, indicated that highest levels of radioactivity were in the liver and kidney cortex. No difference in the pattern of distribution for the two doses was observed. In different groups of rats receiving 20-400 mg/kg bw oral doses of ^{14}C-DBCP, the tissue levels of radiocarbon, of total bound radiocarbon and of total DBCP were analyzed at 6 and 24 hours after dosing (Table III-2). The higher the dose, the higher the concentration of DBCP residue, total radiocarbon and total bound radiocarbon in plasma, blood cells, liver, kidney and testes at 6 hours. By 24 hours, the levels of DBCP had declined to $\leq$0.32 ppm for all tissues in each dose group except for adipose tissue in

TABLE III-2

Tissue Radiocarbon After a Single Oral Dosing of 20-400 mg/kg of ^{14}C-DBCP[a]

Duration (hours)	Dose (mg/kg)	Fraction	ppm Equivalents of ^{14}C-DBCP					
			Plasma	Blood Cells	Liver	Kidney	Testes	Adipose Tissue
6	20	t	3.43 ± 0.65	3.01 ± 0.09	20.9 ± 3.1	30.1 ± 4.3	2.01 ± 0.28	4.52 ± 1.41[b]
		b	2.01 ± 0.72	1.73 ± 0.22	14.7 ± 1.8	12.5 ± 1.8	0.73 ± 0.16	--
		DBCP	0.027 ± 0.12	0.086 ± 0.040	0.034 ± 0.012	0.025 ± 0.016	0.009 ± 0.004	2.14 ± 0.53
	50	DBCP	0.084 ± 0.011	0.326 ± 0.170	0.097 + 0.045	0.146 ± 0.019	0.042 ± 0.026	25.4 ± 7.1
	100	DBCP	0.174 ± 0.071	0.635 ± 0.333	0.166 ± 0.064	0.532 ± 0.293	0.134 ± 0.112	64.3 ± 21.9
	200	t	6.81 ± 1.96	4.85 ± 1.24	48.3 ± 10.4	75.4 ± 16.2	9.54 ± 3.49	--
		b	3.99 ± 1.33	3.47 ± 1.16	33.5 ± 7.2	41.5 ± 12.1	4.30 ± 1.25	--
		DBCP	1.32 ± 1.19	2.67 ± 1.54	3.11 ± 1.70	2.80 ± 2.27	0.39 ± 0.32	149.4 ± 96.9
	400	t	17.32 ± 6.14[c]		120.7 ± 18.6	93.7 ± 20.1	12.7 ± 3.7	--
		b	--		76.1 ± 20.3	65.2 ± 22.3	6.59 ± 2.41	--
		DBCP	1.54 ± 1.49	4.66 ± 4.29	5.33 ± 3.31	4.04 ± 2.49	0.82 ± 0.48	--
24	20	t	0.89 ± 0.11	1.54 ± 0.18	15.64 ± 3.81	9.08 ± 1.64	0.64 ± 0.10	1.46 ± 0.58[b]
		b	0.76 ± 0.15	1.03 ± 0.17	10.42 ± 2.90	6.02 ± 1.70	0.47 ± 0.09	--
		DBCP	0.001 ± 0.000	0.002 ± 0.001	0.001 ± 0.001	0.001 ± 0.000	0.002 ± 0.001	0.30 ± 0.39
	50	DBCP	0.003 ± 0.003	0.039 ± 0.002	0.005 ± 0.008	--	0.031 ± 0.052	3.84 ± 2.51
	100	DBCP	0.015 ± 0.007	0.071 ± 0.004	0.024 ± 0.023	0.066 ± 0.082	0.020 ± 0.002	4.44 ± 0.78
	200	t	16.5 ± 3.1	15.4 ± 1.5	129.8 ± 10.8	138.7 ± 17.5	10.8 ± 1.8	--
		b	10.0 ± 2.4	13.1 ± 1.1	52.7 ± 11.1	61.5 ± 10.2	5.5 ± 0.5	--
		DBCP	0.22 ± 0.06	0.07 ± 0.02	0.32 ± 0.26	0.15 ± 0.12	0.04 ± 0.03	21.5 ± 3.7
	400				dead			

[a] Source: Kato et al., 1980

[b] Values reported previously

[c] Value with whole blood

t = total radiocarbon in tissue; b = radiocarbon bound with macromolecules

Each value represents mean ± S.D. of 4 rats

rats given >20 mg/kg bw. DBCP, however, accounted for only 4-6.5% of the total radioactivity in the kidney, liver and testes (the target organs of DBCP toxicity, see Target Organ Toxicity Section in Chapter V) at 6 hours. Binding of radiocarbon to tissue (kidney, liver and testes) macromolecules amounted to 40-70% of the tissue radiocarbon for all doses at 6 and 24 hours after dosing. The ratio of bound radiocarbon to tissue radiocarbon did not vary significantly with dose and duration post-treatment. Thus, DBCP was rapidly metabolized to a reactive species that binds with tissue macromolecules. Binding sites were not saturated at the doses studied. The low levels of DBCP detected in liver and kidney by Ruddick and Newsome (1979), who analyzed the tissues for parent compound, are consistent with the findings of Kato et al. (1980). No binding of radioactivity was detected in adipose tissue in a previous study by Kato et al. (1979a).

Metabolism

Jones et al. (1979) studied the metabolic fate of DBCP in rats administered an intraperitoneal dose of 50 mg DBCP/kg bw. The DBCP used had been purified to remove epichlorohydrin, a contaminant as well as a metabolite of technical DBCP. A metabolic pathway was proposed, and it was estimated that 85% of the dose was biotransformed and administered in propylene glycol. In this pathway, debromination and hydration of DBCP at 2-C yields the proposed intermediate, 1-bromo-3-chloropropan-2-ol. Further dehalogenation would result in the formation of epibromohydrin and epichlorohydrin, either of which can react with reduced glutathione (GSH) to form, after further metabolism, N-acetyl-S-(2,3-epoxypropyl)cysteine. Reaction of this epoxide with GSH would yield the dimercapturic acid, N,N'-diacetyl-1,3-(bis-cysteinyl)-propan-2-ol. Hydrolysis of the epoxide would result in the formation of the mercapturic acid, N-acetyl-S-(2,3-dihyroxypropyl)cysteine. These mercapturic acids were identified in the urine of rats treated with DBCP, epichlorohydrin or epibromohydrin. Hydrolysis of epibromohydrin and epichlorohydrin would result in α-bromohydrin and α-chlorohydrin, respectively. Dehalogenation would yield 2,3-epoxy-1-propanol, which when reacted with GSH, would yield the mercapturic acid, N-acetyl-S-(2,3-dihydroxylpropyl)-cysteine. The α-halohydrins can also be oxidized to β-halolactic acids, which are reduced to the β-halolactaldehydes. The beta-halolactaldehydes are oxidized to oxalic acid. Methyl β-chlorolactic acid and methyl β-bromolactic acid were identified in the methylated ether extract of the urine of DBCP-treated rats, lending support to the proposed pathway. Kato et al. (1979a) reported that $^{14}CO_2$ was detected in the expired air of rats that had been treated with ^{14}C-DBCP. This finding supports the proposed pathway of Jones et al. (1979), since a 2-carbon compound, oxalic acid, is a proposed metabolite.

Marsden and Casida (1982) recovered an identical percentage (0.037%) of a 2.36 mg/kg bw intraperitoneal dose and a 23.6 mg/kg bw gavage dose of DBCP as 2-bromoacrylic acid in the 24-hour urine of rats. The investigators suggested that 2-bromoacrolein was an in vivo intermediate. The in vitro metabolism of DBCP by rabbit liver microsomes in the presence of NADPH also resulted in the formation of 2-bromoacrylic acid. A pathway was proposed whereby DBCP is converted to 2-bromo-3-chloro-propanaldehyde or 2,3-dibromopropanaldehyde, either of which may be dehydrogenated and dehalogenated to 2-bromoacrylic acid. The low levels of 2-bromoacrylic acid recovered in the urine may have been due to high reactivity of these compounds. It was suggested that the mutagenic activity of DBCP with metabolic activation (see

Mutagenicity Section in Chapter V) may be due, in part, to 2-bromoacrolein, a potent mutagen (Rosen et al., 1980).

Epichlorohydrin, epibromohydrin, α-chlorohydrin and α-bromohydrin were all mutagenic without metabolic activation in Salmonella typhimurium strain TA100 (Stolzenberg and Hine, 1979) and may account for part of the mutagenic action of DBCP (see Mutagenicity Section in Chapter V).

DBCP administration depleted the GSH content of the liver and kidney of rats and mice (Kato et al., 1980; Kluwe et al., 1981). The depletion of GSH was dose-related and coincided in time with the dose-related increased binding of radioactive metabolites of ^{14}C-DBCP to liver and kidneys, which resulted in toxicity (Kato et al., 1980). The depletion of GSH was enzyme dependent in in vitro studies (Kluwe et al., 1981). This mechanism of toxicity of DBCP is discussed more fully in Chapter VII.

The depletion of GSH by metabolites of DBCP is consistent with the metabolic pathway proposed by Jones et al. (1979). According to the scheme, GSH can react with 4 separate epoxide intermediates, namely, epibromohydrin, epichlorohydrin, 2,3-epoxypropan-1-ol and N-acetyl-S-(2,3-epoxypropyl)-cysteine.

Excretion

Information on the excretion of DBCP is sparse. Jones et al. (1979) found that rats eliminated 9% of the dose of 50 mg DBCP/kg bw in the expired air, 2 hours after intraperitoneal injection. An additional 5% was expired in the next 2 hours. No unchanged DBCP was eliminated in the urine, but mercapturic acid conjugates were detected in urine.

Kato et al. (1979a) administered ^{14}C-DBCP orally to male Wistar rats at doses of 20-400 mg/kg bw and observed that nearly 85% of the radioactivity was eliminated in the urine, bile and expired air. No unchanged DBCP was eliminated in the urine, and only traces of the eliminated label in the expired air were unchanged DBCP. Urinary and fecal excretion accounted for 51.4 and 22% of the radioactivity after 14 days, while 17.6% was excreted in the expired air after 48 hours, and biliary excretion accounted for 22.7% of the dose in 24 hours. The urine was the predominant route of elimination for metabolites of DBCP.

Detectable levels of ^{14}C-DBCP and high levels of total radioactivity and bound radioactivity were still found in plasma, blood cells, liver, kidney and testes of rats 24 hours after single oral doses of 20-200 mg/kg bw ^{14}C-DBCP (Kato et al., 1980). The adipose tissue contained a relatively high level (21.5 ppm) of unchanged DBCP at 6 and 24 hours after the rats received 200 mg/kg bw.

Summary

DBCP is absorbed by the GI tract, the lungs and the skin of laboratory animals as evidenced by reports of systemic toxicity and distribution of the pesticide after administration by these routes. Following absorption, DBCP is widely distributed in 1-3 hours to tissues throughout the body of rats (Ruddick and Newsome, 1979), where reactive intermediate metabolites bind to tissue macromolecules (Kato et al., 1980). No binding to macromolecules was found in adipose tissue, which accumulated the highest levels of unchanged DBCP compared with other tissues. Decline of tissue levels of DBCP, with the exception of adipose tissue, was complete in 12-24 hours (Ruddick and

Newsome, 1979; Kato et al., 1980), while the concentrations of total tissue metabolites and bound metabolites continued to increase at 24 hours after dosing with 200 mg DBCP/kg bw (Kato et al., 1980). As compared with levels in other tissues, relatively high levels of parent DBCP compared with other tissue persisted in adipose tissue for 24 hours (Ruddick and Newsome, 1979; Kato et al., 1980). Low levels of DBCP were found in the fetuses of pregnant rats that had been treated with DBCP, indicating that transplacental transfer had occurred (Ruddick and Newsome, 1979).

Metabolites identified in the urine of rats that had been treated with DBCP included β-chloroacetic acid and β-bromolactic acid and the mercapturic acids, N,N'-diacetyl-1,3-(bis-cysteinyl)propanol and N-acetyl-S-(2,3-dihydroxylpropyl)cysteine (Jones et al., 1979). A proposed metabolic pathway was discussed. The finding of mercapturic acids indicated that intermediates had reacted with GSH. Administration of DBCP has resulted in the depletion of GSH in liver, kidney and testes (Kato et al., 1980; Kluwe et al., 1981) of rats. A small amount of 2-bromoacrylic acid was detected in the urine of rats after DBCP administration and was formed from DBCP _in vitro_ by rabbit liver microsomes (Marsden and Casida, 1982). An alternate pathway, involving the intermediate 2-bromoacrolein, was proposed.

Information regarding the excretion of unchanged DBCP is limited to the observations that expiration of 14% of an intraperitoneal dose of 50 mg DBCP/kg bw in rats occurs in 4 hours (Jones et al., 1979), while an oral dosing of 20 mg/kg bw results in only 0.04% unchanged compound expired in 48 hours (Kato et al., 1979a). Radioactivity from ^{14}C-DBCP was retained in most tissues for at least 24 hours (Kato et al., 1980).

IV. HUMAN EXPOSURE

This chapter will be submitted by the Science and Technology Branch, Criteria and Standards Division, Office of Drinking Water.

V. HEALTH EFFECTS IN EXPERIMENTAL ANIMALS

General Toxicity

Effects of Acute and Short-Term Exposure. Acute LD_{50} and LC_{50} values for DBCP by various routes of exposure in various species are presented in Table V-1. Reported acute oral LD_{50} values varied between and within species as follows: from 170-350 mg/kg bw in rats, 260-410 mg/kg bw in mice, 210-316 mg/kg bw in guinea pigs and 180-440 mg/kg bw in rabbits (Torkelson et al., 1961; Rakhmatullayev, 1971). DBCP was more toxic to rabbits when applied in propylene glycol (LD_{50} = 500 mg/kg bw) than when applied undiluted to the shaven skin (LD_{50} = 1400 mg/kg bw). Loss of DBCP from the skin from evaporation was prevented by a rubber sleeve (Torkelson et al., 1961). Torkelson et al. (1961) also determined the LC_{50} values at 1, 2, 4 and 8 hours of inhalation exposure (see Table V-1). When administered intraperitoneally to mice, DBCP was slightly less toxic to prepubertal CD-1 mice than to young adult CD-1 mice (Lee and Suzuki, 1979).

Many of the studies employing acute oral exposures to DBCP were designed to investigate biochemical changes and mechanisms of toxicity (discussed in Chapter VII) to testis, kidney and liver of rats (Table V-2). The single oral doses, administered by gavage, were generally high and were near or within the range of reported LD_{50} values (Tofilon et al., 1980; Suzuki and Lee, 1981; Moody et al., 1980, 1982a,b). Rats were killed at various time intervals after dosing in order to study the particular parameter of concern, and no mention was made of outward manifestations of toxicity or mortality. Doses of 100-300 mg DBCP/kg bw to Sprague-Dawley rats resulted in decreased levels of hepatic, renal and testicular microsomal cytochrome

TABLE V-1

Acute LD_{50}/LC_{50} Values for DBCP

Route	Species/Strain	Sex/No.	Weight/Age	Purity	Vehicle	LD_{50}/LC_{50}[a]	Comment	Reference
Oral	rat/NR	M/NR	NR/NR	95-98%	NR	170 mg/kg bw (150-200 mg/kg bw)	Lab A[b]	Torkelson et al., 1961
Oral	rat/NR	M/NR	NR/NR	95-98%	NR	300 mg/kg bw (230-370 mg/kg bw)	Lab B[c] kidney degeneration was observed after treatment	Torkelson et al., 1961
Oral	rat/NR	NR/NR	NR/NR	NR	NR	350 mg/kg bw (NR)	NC	Rakhmatullayev, 1971
Oral	mouse/NR	F/NR	NR/NR	95-98%	NR	260 mg/kg bw (NR)	Lab A[b]	Torkelson et al., 1961
Oral	mouse/NR	F/NR	NR/NR	95-98%	NR	410 mg/kg bw (270-620 mg/kg bw)	Lab B[c]	Torkelson et al., 1961
Oral	guinea pig/NR	M/NR	NR/NR	95-98%	NR	210 mg/kg bw (150-300 mg/kg bw)	Lab B[c]	Torkelson et al., 1961
Oral	guinea pig/NR	NR/NR	NR/NR	NR	NR	316 mg/kg bw (NR)	NC	Rakhmatullayev, 1971
Oral	rabbit/NR	M/NR	NR/NR	95-98%	NR	180 mg/kg bw (NR)	Lab B[c]	Torkelson et al., 1961
Oral	rabbit/NR	NR/NR	NR/NR	NR	NR	440 mg/kg bw (NR)	NC	Rakhmatullayev, 1971
Inhalation	rat/Long-Evans	M+F/NR	NR/NR	95-98%	NR	3557 mg/m³[d] (NR) 2242 mg/m³ (1952-2561 mg/m³) 1488 mg/m³ (1305-1710 mg/m³) 955 mg/m³ (870-1140 mg/m³)	1 hour Lab A[b] 2 hours Lab A 4 hours Lab A 8 hours Lab A	Torkelson et al., 1961
Dermal	rabbit/albino	NR/NR	NR/NR	95-98%	none	1400 mg/kg bw (800-2500 mg/kg bw)	applied to shaven skin for 24 hours under rubber sleeve Lab A[b]	Torkelson et al., 1961; Kodama and Dunlap, 1956

TABLE V-1 (cont.)

Route	Species/Strain	Sex/No.	Weight/Age	Purity	Vehicle	LD_{50}/LC_{50}[a]	Comment	Reference
Dermal	rabbit/albino	NR/NR	NR/NR	95-98%	propylene glycol	500 mg/kg bw (340-750 mg/kg bw)	applied to shaven skin for 24 hours under rubber sleeve Lab A[b]	Torkelson et al., 1961
i.p.	mouse/ICR	M/4/group	15-20 g/NR	99.3%	corn oil	175 mg/kg bw (158-192 mg/kg bw)	NC	Kluwe et al., 1981
i.p.	mouse/CD-1	M/5-10/ group	NR/22±2 days	>99%	corn oil	180.7 mg/kg bw (154.0-197.5 mg/kg bw)	MTD = 100 mg/kg bw	Lee and Suzuki, 1979
i.p.	mouse/CD-1	M/5-10/ group	25±3 g/ 8-9 weeks	>99%	corn oil	123.0 mg/kg bw (100.5-141.5 mg/kg bw)	MTD = 80 mg/kg bw	Lee and Suzuki, 1979

[a] Values in parentheses are 95% confidence limits.

[b] Lab A - Department of Pharmacology and Experimental Therapeutics, University of California School of Medicine, San Francisco, CA.

[c] Lab B - Biochemical Research Laboratory of Dow Chemical Company, Midland, MI.

[d] Concentrations in mg/m^3 were calculated from concentrations expressed as ppm.

NR = Not reported; NC = No comment; MTD = Minimum toxic dose; M = male; F = female

TABLE V-2 (cont.)

Route	Species/Strain	Sex/No.	Weight/Age	Purity	Vehicle	Dose/ Concentration	Duration	Effect	Reference
Oral	rat/Sprague-Dawley	M/NR	200-250 g/ NR	NR	mineral oil	0, 628 mg/kg bw	single dose	Significant increase in relative content of palmitic and linoleic acids, significant decrease in content of stearic and arachidonic acid in liver microsomes. Cytochrome P-450 content reduced to 76% of controls.	Moody et al., 1980
Oral	rat/Wistar	M/4	NR/9 weeks	pure (prepared in lab)	olive oil	400 mg/kg bw	single dose	100% mortality in 24 hours. Degeneration and focal necrosis of centrilobular hepatocytes and degeneration of renal tubules. Pretreatment of rats with phenobarbital enhanced the severity of necrosis and degeneration, SKF-525A or GSH pretreatment protected rats from liver necrosis.	Kato et al., 1980
Oral	rat/NR	M+F/NR	158-198 g/ NR	97%	vegetable oil	0, 100 mg/kg bw	single dose	Reduced spermatogenesis and decreased sperm count and motility in treated males, atypical estrus cycles in treated females.	Reznik and Sprinchan, 1975
Oral	rat/F344	M/6/ group	230-260 g/ NR	>99%	corn oil	0, 40 mg/kg bw/day	4 days	Reduced body weight at 3 days, watery stool, significantly increased BUN compared with controls ($p<0.05$) and compared with i.p. ($p<0.05$); significantly decreased urine specific gravity compared with control ($p<0.05$). Significantly increased kidney-to-brain weight ratio compared to control ($p<0.05$) and i.p. ($p<0.05$). Histology: kidney - necrotic proximal tubule cells; liver - hepatocellular swelling; testes - disruption of seminiferous tubule structure, caput epididymis had severely degenerating epithelial cells and decreased sperm density. Similar effects were observed when DBCP was administered i.p. and s.c. (see Table V-2 entries below for this investigator.	Kluwe, 1981a

TABLE V-2 (cont.)

Route	Species/Strain	Sex/No.	Weight/Age	Purity	Vehicle	Dose/ Concentration	Duration	Effect	Reference
Oral	rat/Sprague Dawley	M/15/ group	NR/11 weeks	>95%	olive oil	0, 50 mg/kg bw/day	5 days	Testicular degeneration in 5/15 treated rats, the effect was not further discussed.	Saito-Suzuki et al., 1982
Oral	rat/Wistar	F/15/ group	175-200 g/ NR	NR	corn oil	0, 12.5, 25.0 and 50.0 mg/kg bw/day	10 days (day 6-15 of gestation)	Rats were necropsied on day 22 of gestation. Decreased mean body weight at 25 and 50 mg/kg ($p<0.05$ and $p<0.001$, respectively) compared with controls. One dam receiving 50 mg/kg/day died on day 17. The 50 mg/kg/day dose was toxic to fetuses, as well. No maternal toxicity or teratogenicity was observed at 12.5 mg/kg bw/day.	Ruddick and Newsome, 1979
Inhalation	rat/NR	NR/NR	NR/NR	95-98%	air	483, 966, 1836 and 2803 mg/m^{3a}	0-7 hours	Permanent scarring of kidneys at 483 mg/m^3 deaths were common. LAB B^b	Torkelson et al., 1961
Inhalation	rat/Long-Evans	M+F/NR	NR/NR	98-98%	air	≥580 mg/m^3	1-7 hours	Pulmonary irritation, moderate CNS depression, apathy, sluggishness, ataxia, deaths 72 hours. LAB A^c	Torkelson et al., 1961; Kodama and Dunlap, 1956
Inhalation	rat/Long-Evans	M+F/NR	NR/NR	NR	air	1305-6475 mg/m^3	"acute exposure"	Pulmonary irritation and liver damage, minimum hypnotic effect	Kodama and Dunlap, 1956
Inhalation	rat/Sprague-Dawley	M/19	NR/NR	purified in lab	air	0, 2.9, 9.7, 29 and 77 mg/m^3	14 days continous exposure	At <9.7 mg/m^3, no changes in testes at 1 or 15 days postexposure; at 29 mg/m^3, slight damage (decreased number of germ cells or atrophy of a few seminiferous tubules at day 1 and 15 in 1 of 2 rats); at 77 mg/m^3, testicular lesions were severe with necrotic germ cells and severely atrophied tubules.	Saegusa et al., 1982

TABLE V-2 (cont.)

Route	Species/Strain	Sex/No.	Weight/Age	Purity	Vehicle	Dose/ Concentration	Duration	Effect	Reference
Inhalation	rat/Sprague-Dawley	M/11	NR/NR	purified in lab	air	97 mg/m³	14 days continous exposure	Rats killed at 1, 16, 36 days and 1 year postexposure. Histology: testes - atrophied seminiferous tubules containing only Sertoli cells, necrotic germ cells at day 1, atrophy progressed to severe aspermatogenesis, 1 rat died spontaneously at 1 year and had a hemangioma in testes. Kidney - some necrosis of proximal tubule at day 1, giant epithelial cells at days 1 and 16; at 1 year, 1 rat had a greatly enlarged right kidney with an adenocarcinoma metastisizing to left kidney, lung, thymus and abdomen. Lungs - bronchial dilation, emphysematous aleovar distention, giant epithelial cells at days 1 and 16 and necrotic epithelial cells of bronchioles at day 1, but not at day 16. Spleen - atrophy of white pulp and decreased lymphocytes in red pulp at day 1 only.	Saegusa et al., 1982
Dermal	rabbit/NR	NR/NR	NR/NR	95-98%	none	0.5 mℓ (1.05 g/rabbit)	single application or 20 repeated applications	For single dose - slight erythema to abraded area. For repeated doses - slight crustiness, extensive necrosis of dermis and subcutaneous tissue of 1 rabbit	Torkelson et al., 1961
Dermal	rabbit/NR	NR/NR	NR/NR	95-98%	dipropyglycol, methyl ether	10% solution	1 application/ day for 10 days	Slight hyperemia and scaliness	Torkelson et al., 1961
Ocular	rabbit/NR	NR/NR	NR/NR	95-98%	propylene glycol or none	1% solution or undiluted	single application	Slight pain and irritation of conjunctiva and iris lasting 1-2 days	Torkelson et al., 1961
i.p.	mouse/ICR	M/4/ group	15-20 g/NR	99.3%	corn oil	0, 33, 100, 330 or 1000 mg/kg bw	single dose	Significant dose-related decrease in hepatic and renal GSH; lung, stomach and testicular GSH also reduced; at ≥100 mg/kg bw, there was severe narcosis.	Kluwe et al., 1981

TABLE V-2 (cont.)

Route	Species/Strain	Sex/No.	Weight/Age	Purity	Vehicle	Dose/ Concentration	Duration	Effect	Reference
i.p.	rat/F344	M/4/ group	150 g/NR	99.3%	corn oil	0, 100, 250, 330 or 1000 mg/kg bw	single dose	Significant dose-related decrease in hepatic, renal, lung, stomach and testis GSH. All rats died at 1000 mg/kg bw.	Kluwe et al., 1981
i.p.	rat/F344	M/6/ group	230-260 g/ NR	>99%	corn oil	0, 40 mg/kg bw/day	4 days	Decreased body weight at 3 days, watery stools, significantly elevated BUN and decreased specific gravity of urine compared with controls ($p<0.05$). No change in kidney-to-brain weight ratio. Qualitatively similar histopathologic lesions in kidney, liver, testis and caput epididymis as seen when DBCP was administered orally or s.c. for 4 days (see entries in this Table above and below for this investigator).	Kluwe, 1981a
s.c.	rat/F344	M/6/ group	230-260 g/ NR	>99%	corn oil	0, 10, 20, 40, 80 or 120 mg/kg bw	single dose	No effect on body weight gain and no clinical signs at <40 mg/kg; at 80 mg/kg, rats did not gain weight; at 120 mg/kg - net body weight loss, disheveled fur, hypothermia, diarrhea, elevated BUN and serum creatinine content; at 80 and 120 mg/kg - increased urinary excretion of glucose. Increased urinary protein excretion, urine volume and urine specific gravity at ≥40 mg/kg. Increased urinary excretion of GOT, LDH, N-acetyl-B-D-glucosamidase, alkaline phosphatase; gamma-glutamyl-transpeptidase and NA^+ and K^+. Increased serum GPT, sorbitol dehydrogenase, LDH and alkaline phosphatase at 80 and 120 mg/kg. At ≥40 mg/kg, moderate to severe cytoplasmic vacuolization of proximal tubular epithelium becoming more severe with increasing dose. Mild hepatocellular swelling at ≥40 mg/kg progressing to hepatocellular necrosis at 120 mg/kg. Reduced sperm counts and epithelial degeneration of caput epididymis at 80 and 120 mg/kg.	Kluwe, 1981a

TABLE V-2 (cont.)

Route	Species/Strain	Sex/No.	Weight/Age	Purity	Vehicle	Dose/ Concentration	Duration	Effect	Reference
s.c.	rat/F344	M/6/ group	230-260 g/ NR	>99%	corn oil	0, 40 mg/kg bw/day	4 days	Decreased body weight at 3 days, watery stools, significantly elevated BUN compared with controls ($p<0.05$) with i.p. route ($p<0.05$) and with oral route ($p<0.05$). Significantly decreased urine specific gravity compared with control ($p<0.05$) and with i.p. route ($p<0.05$). Significantly increased kidney-to-brain weight ratio compared with control ($p<0.05$) compared with i.p. route ($p<0.05$) and compared with oral route ($p<0.05$). Qualitatively similar histopathologic lesions in kidney, liver, testis and caput epididymis to those observed after oral or i.p. administration. Most severe by s.c. (see entries in this Table above for oral and i.p. routes for this investigator.)	Kluwe, 1981a
s.c.	rat/F344	M/10/ group	6 days 25 days	>98%	corn oil	0, 20, 40, 80, 160 mg/kg bw/day	1 day	Doses ≥ 20 mg/kg reduced body and gonadal weight gains ($p<0.05$) and caused hypospermatogenesis or seminiferous tubular atrophy in 6-day-old rats killed at 120 days of age. Doses below 160 mg/kg were ineffective in 25-day-old rats.	

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[a] Concentrations in mg/m³ were calculated from concentrations expressed as ppm:

$$mg/m^3 = \frac{ppm \times MW}{24.45}$$

[b] LAB B - Biochemical Research Laboratory of Dow Chemical Co., Midland, MI.

[c] LAB A - Department of Pharmacology and Experimental Therapeutics, University of California School of Medicine, San Francisco, CA.

NR = Not reported; M = male; F = female

P-450 (Moody et al., 1982a, Tofilon et al., 1980). The loss of cytochrome P-450 coincided with decreased heme levels and increased activity of delta-aminolevulinic acid synthetase (Tofilon et al., 1980). These changes were transient with return to control levels in 6 days.

Liver weights, liver protein content and liver RNA content were increased above control levels, but liver damage was minimal (Moody et al., 1982b). Moody et al. (1980) also found changes in the lipid components of hepatic microsomes at a very high acute oral dose (628 mg/kg bw) to male Sprague-Dawley rats. Suzuki and Lee (1981) reported increased activities of the microsomal enzymes: aryl hydrocarbon hydroxylase was increased in the kidney, testes, and stomach, and epoxide hydrolase was increased in liver, kidneys and testes of rats treated orally with 125 mg/kg bw DBCP. Kato et al. (1980) administered a lethal oral dose of DBCP to rats and found necrosis of centrilobular hepatocytes and degeneration of renal tubules. Pretreatment of the rats with phenobarbital (an inducer of microsomal enzymes) resulted in more severe lesions. Pretreatment with SKF-525A (an inhibitor of some mixed function oxidases) or with GSH, protected the rats from the hepatotoxicity of DBCP (or metabolite, see Metabolism Section in Chapter III). In a study from the Russian literature, it was reported that a single oral dose of 100 mg DBCP/kg bw to rats resulted in reduced spermatogenesis in males and alterations in the estrus cycle of females (Reznik and Sprinchan, 1975). Effects of repeated oral doses of DBCP at lower levels have been reported (see Table V-2; Saito-Suzuki et al., 1982; Ruddick and Newsome, 1979; Kluwe, 1981a). Testicular degeneration was observed in 5/15 of 15 male rats treated with DBCP by gavage at 50 mg/kg bw/day for 5 days (Saito-Suzuki et al., 1982). Ruddick and Newsome (1979) reported a no-effect level of 12.5 mg/kg bw/day of DBCP for 10 days (days 6-15 of gestation) in rat dams and fetuses, while 25 and 50 mg/kg/day doses resulted in significantly decreased mean body weight in treated dams compared with controls. This study is discussed more fully in the Teratogenicity and Other Reproductive Effects Section.

Gross and histopathologic signs of toxicity were reported in F344 rats receiving 4 daily doses of 40 mg/kg bw/day by gavage (Kluwe, 1981a). The signs included decreased body weight, watery stools, elevated blood urea nitrogen and histologic damage to kidneys, liver and testes. Similar effects were observed when an identical dose was administered intraperitoneally or subcutaneously (see Table V-2 for intraperitoneal and subcutaneous routes). The effects were generally less severe following intraperitoneal dosing and somewhat more severe following subcutaneous injection than when DBCP was administered by gavage. For these reasons, Kluwe (1981a) studied the dose-response of DBCP in rats following single subcutaneous injections. Groups of six male F344 rats were injected with 0, 10, 20, 40, 80 or 120 mg DBCP/kg bw. The results are presented in Table V-2. The no-effect level for single subcutaneous injection of DBCP was 20 mg/kg bw in this study.

The effects of subcutaneous exposure to DBCP (100 mg/kg bw) on kidney function were identical to those reported previously. The effects were transient, returning to normal levels in 5-10 days after exposure. Histologic damage was still evident, however, 30 days postexposure (Kluwe, 1981b) (30-day results were not included in Table V-2), since duration exceeded the arbitrary 14 days for an acute study. Effects on the testis persisted for at least 30 days. Effects on the liver were transient with return of serum enzyme levels to control and reversal of hepatocellular swelling occurring in 48-78 hours.

Toxic effects of DBCP administered to rats by the inhalation route, either acutely or for 14 days, are summarized in Table V-2. For the acute exposures (0-7 hours duration), concentrations of DBCP in air ranging from 50-670 ppm (483-6475 mg/m³) resulted in kidney and liver damage, delayed deaths, pulmonary irritation and CNS depression (Torkelson et al., 1961; Kodama and Dunlap, 1956). Continuous exposure for 14 days of 3 and 8 ppm (29 and 77 mg/m³) DBCP resulted in slight and severe damage to testes, respectively (Saegusa et al., 1982). At concentrations of $\leq$1 ppm (9.7 mg/m³) DBCP, no effects on the testes were noted. At 10 ppm (97 mg/m³) DBCP for 14 days, histopathologic damage to the testes, kidneys and lungs was severe (see Table V-2).

Single or repeated dermal or ocular applications of DBCP to rabbits resulted in such local effects as erythema, crustiness, scaliness and irritation to the conjunctiva and iris (Torkelson et al., 1961; see Table V-2).

Intraperitoneal doses of 33-1000 mg/kg bw DBCP to ICR mice or F344 rats resulted in dose-related decreases in hepatic, renal, testicular, pulmonary and stomach nonprotein sulhydryl content (mostly GSH) (Kluwe et al., 1981;

see Table V-2). This effect was transient in liver and epididymis in rats (the time course was not studied in mice) injected subcutaneously with 80 mg/kg bw DBCP; return to control levels occurred in 4-6 hours (Kluwe et al., 1982; data not included in Table V-2). In this study, nonprotein sulfhydryl (GSH) content of kidney and testis was not decreased, but rose above control levels in liver, kidney and testis in 16-48 hours after exposure.

Effects of Subchronic and Chronic Exposure.

Oral Exposure -- Studies on the effects of subchronic and chronic oral exposure of rodents to DBCP are summarized in Table V-3 and Table V-4, respectively.

Subchronic Studies. A subchronic toxicity study with DBCP (99.3-99.71% pure) in male Sprague-Dawley rats has been done by Heindel et al. (1983). Treatment began when the rats were 11.5-12.5 weeks old. Twenty animals per group were given tap water alone (controls) or 200, 100, 50 or 5 ppm solutions of DBCP in drinking water for 64 days. At the end of treatment, the rats were sacrificed. Blood was collected every 2 weeks during the study for clinical chemistry and gonadotropin analysis. Liver, kidney, prostate, epididymus, seminal vesicles and testes were weighed. Epididymal sperm counts and testicular testosterone levels were estimated. Liver, kidney and testes were microscopically examined.

Fresh DBCP solutions were given to the animals weekly, and DBCP was stable with negligible loss in drinking water during this time. Water consumption was decreased ($p<0.05$) in a dose-dependent manner in all treated groups. However, cumulative DBCP intake of 243, 153, 91 and 11 mg DBCP by

TABLE V-3

Subchronic Oral Exposure Effects of Rats and Mice to DBCP

Species/Strain	Sex/No.	Weight/Age	Purity	Vehicle	Dose or Concentration	Duration	Effects	Reference
Mouse/B6C3F$_1$	M+F/5/ sex/group	NR/5 weeks	>90% with 16 minor impurities	corn oil	0, 71, 114, 179, 284 and 451 mg/kg bw/day[a]	6 weeks, followed by 2 weeks observation	100% mortality at ≥284 mg/kg bw/day dose level. At ≤179 mg/kg bw/day, one male and one females died; all others survived. There was no difference in mean body weight gain in any group compared with controls. Clinical signs of toxicity and histopathological observation were not performed.	NCI, 1977
Rat/Osborne-Mendel	M+F/5/ sex/group	NR/6-7 weeks	>90% with 16 minor impurities	corn oil	0, 18, 29, 45, 71 and 114 mg/kg bw/day[a]	6 weeks, followed by 2 weeks observation	No deaths at ≤29 mg/kg bw/day in males, or at 18 mg/kg bw/day in females. Deaths occurred at higher dose levels, but mortality data for specific dose levels were not reported. There was decreased mean body weight gain (22% for males, 33% for females) at 18 mg/kg bw/day. Clinical signs of toxicity or histopathological observations were not performed.	NCI, 1977
Rat/NR	M/10/ group	NR/NR	97-98%	sunflower oil	0, 70 mg/kg bw/day	45 days	5 rats died (it was unclear whether these were all treated rats). The treated rats lost weight and were lethargic. Other effects included decreased hemoglobin content, red and white cell counts; immature neutrophils with reduced phagocytic activity; reduced serum cholinesterase activity; increased prothrombin time. At necropsy internal organs were pale, livers were yellow. Histopathologic changes included hepatocellular necrosis with regeneration, bile duct proliferation, testicular necrosis with regeneration, necrosis of red and white pulp of spleen, cytomegaly of nephron epithelial cells.	Rakhmatullayev, 1969; Faydysh et al., 1970
Rat/Sprague-Dawley	M/20/ group	11.5-12.5 weeks	99.3-99.71%	drinking water	0, 5, 50, 100 or 200 ppm	64 days	Body weight gain was slower in the highest dose group; dose-dependent increase in OCT serum levels suggesting minimal evidence of liver injury. No effect on organ weights at all doses; histology was unremarkable.	Heindel et al., 1983

TABLE V-3 (cont.)

Species/Strain	Sex/No.	Weight/Age	Purity	Vehicle	Dose or Concentration	Duration	Effects	Reference
Rat/Sprague-Dawley	M/4/group	11.5-12.5 weeks	99.3-99.71%	drinking water	100 or 200 ppm	5 days	Glucose, protein and specific gravity of urine were increased at both doses.	Meindel et al., 1983
Rat/NR	M/10/ group	NR/NR	NR	NR	0, 17.5, 35 and 70 mg/kg bw/day	2.5 months	5/10 rats treated at 70 mg/kg bw/day died during exposure period; at 70 mg/kg bw/day dose level, rats had low hemoglobin content and low red and white cell counts; prolonged prothrombin time, high urinary coproporhyrin content and histological damage to livers and kidneys. Reduced phagocytic activity of neutrophils was observed at all dose levels.	Rakhmatullayev, 1971
Rat/Long-Evans	M+F/10 controls/ sex, 14 treated/ sex/group	M/105-145 g F/80-125 g/ NR	95-98%	diet	0, 5, 20, 50, 150, 450 or 1350 mg/kg diet (0, 0.5, 2.0, 5.0, 15.0, 45.0 or 135 mg/kg bw/day)^b	90 days	At $\leq$150 mg/kg diet there were no clinical signs of toxicity. One male rat at the 50 mg/kg diet level died with pneumonia. At 1350 mg/kg diet, rats did not thrive well, were listless and experienced muscular weakness. 4 males and 2 females at this level died. The dead rats had discolored livers, kidneys and stomachs and edema of intestines. Body weight gain was significantly depressed in females treated at $\geq$150 mg/kg diet levels and in males at 450 and 1350 mg/kg diet levels ($p<0.05$). Kidney-to-body weight ratios were significantly increased in female rats at $\geq$20 mg/kg diet and in males at 1350 mg/kg diet levels ($p<0.05$). The liver-to-body weight ratios were significantly increased in females at 450 mg/kg diet and in males and females at 1350 mg/kg diet levels ($p<0.05$). Cloudy swelling of the liver was observed in 5 treated males at unspecified dietary levels. Kidney histopathology was unremarkable.	Torkelson et al., 1961; Kodama and Dunlap, 1956

TABLE V-3 (cont.)

Species/Strain	Sex/No.	Weight/Age	Purity	Vehicle	Dose or Concentration	Duration	Effects	Reference
Rat/Sprague-Dawley	M+F/10 each/group	NR/60 days	97.3%	drinking water	0, 0.02, 0.2, 2.0 and 20.0 mg/kg bw/day	60 days prior to mating for both sexes; followed by 10 days exposure during mating for males and throughout mating, gestation and 5 days of lactation for females.	No deaths, clinical or other observed toxicity in the three lowest dose groups. Food and water intake were markedly decreased at the high dose. Significant ($p<0.05$) body weight loss in both sexes at the high dose. Relative organ weights were also affected in both sexes (see text). Significantly ($p<0.05$) reduced pup weights at the high dose at 1 and 4 days after birth. No treatment-related pathology was found.	Johnston et al., 1981
Rat/NR	M+F/NR	125-141 g/NR	97%	vegetable oil	0 and 10 mg/kg bw	4-5 months multiple doses, NOS	In males, the sperm count became progressively and significantly more reduced with time compared with controls ($p<0.001$ at 4 months). Sperm motility was decreased. Spermatogenesis was reduced. Biochemical effects included increased concentrations of acid and alkaline phosphate and activity of hyaluronidase in testes. At 5 months, 70% of the treated females had atypical estrus cycles.	Reznik and Sprinchan, 1975

TABLE V-3 (cont.)

Species/Strain	Sex/No.	Weight/Age	Purity	Vehicle	Dose or Concentration	Duration	Effects	Reference
Rat/NR	M/7/group	NR/NR	NR	NR	0, 0.005, 0.05, 0.5 or 5 mg/kg bw/day	8 months	No effects on body weight in any test group. There was a mg/kg bw/day dose-related increase in urinary coproporphyrin content. No effects were observed at 0.005 mg/kg bw level. At the 0.05 mg/kg bw level, effects on the phagocytic activity of neutrophils, conditioned reflexes and gonadal "generative function" (not further defined) were reported. At 0.5 and 5 mg/kg bw, effects included decreased blood cell counts and hemoglobin content, increased BUN concentration, proteinurea, decreased testes weight, decreased sperm motility, histopathology of testes (not further described). The incidences, severity and levels of significance of any of these effects were not reported.	Rakhmatullayev, 1971

[a] The actual experimental doses were 100, 160, 251, 398 and 631 mg/kg bw/day for 5 days/week for mice and 25, 40, 63, 100 and 160 mg/kg bw/day for 5 days/week for rats. The doses presented in the table were calculated by multiplying the experimental doses by 5 days ÷ 7 days.

[b] See text

NR = Not reported; NOS = not otherwise specified; M = male; F = female

TABLE V-4

Toxic Effects of Chronic Oral Exposure of Rats and Mice

Species/Strain	Sex/No.	Weight/Age	Purity	Vehicle	Dose^{a,b} (mg/kg bw/day)	Duration	Effects	Reference
Rat/Osborne-Mendel					0	109 weeks observation	Dose-related decrease in mean body weight from 5 months, hunched appearance of treated rats beginning at 14 weeks, becoming progressively more prevalent (80% by 34 weeks). At week 34, 15% low-dose, 50% high-dose rats had abdominal urine stains. Statiscally significant increased mortality ($p<0.001$): By week 62, 80% high-dose males died. By week 83, 90% of low-dose males died. In male vehicle control, 10 were killed at week 64, 2 were killed at week 83, others died spontaneously by week 83, 55% of untreated control survived 88%. Early mortality may be tumor-related (see Oral Exposure Section under Carcinogenicity). The incidence of toxic tubular nephropathy: 49/50 high-dose, 50/50 low-dose, 0/40 control.	NCI, 1977
	M/20 untreated 20 vehicle controls	NR/6-7 weeks	>90% 16 minor impurities	corn oil	0 (vehicle)	78 weeks vehicle 5 weeks observation		
	50 treated per group				corrected TWA 10.7	78 weeks treated 5 weeks observation		
					corrected TWA 20.7	64 weeks treated		
	F/20 untreated, 20 vehicle controls	NR/6-7 weeks			0	109 weeks observation	Dose-related decrease in mean body weight from 5 months, hunched appearance of treated rats beginning at 14 weeks, becoming progressively more prevalent (80% by 34 weeks). At 34 weeks, 15% low-dose and 50% high-dose rats had abdominal urine stains. Significant dose-related increased mortality ($p<0.001$). By week 61, 94% of high-dose females died; by week 73, 84% of low-dose died. In vehicle control, 10 killed at week 64, 5 killed at week 83, others died spontaneously by week 77. 65% of untreated control survived 109 weeks. Early mortality may be tumor-related (see Oral Exposure Section under Carcinogenicity). Incidence of toxic tubular nephropathy: 44/44 high-dose, 42/42 low-dose, 0/40 control.	
					0 (vehicle)	78 weeks vehicle 5 weeks observation		
	50 treated per group				corrected TWA 10.7	73 weeks treated		
					corrected TWA 20.7	64 weeks treated		

TABLE V-4 (cont.)

Species/Strain	Sex/No.	Weight/Age	Purity	Vehicle	Dose^{a,b} (mg/kg bw/day)	Duration	Effects	Reference
Rat/Charles River CD	M/60/group	92-143 g/ 5-6 weeks	95%	diet	0	104 weeks, with an interim kill of 10 rats/ sex/group at 52 weeks	30% mortality, exclusive of interim kill.	Hazelton Laboratories, 1977; U.S. EPA (44 FR 65135)
					0.2		30% mortality, exclusive of interim kill.	
					0.68		40% mortality, exclusive of interim kill.	
					2.0		62% mortality, exclusive of interim kill. At 52 weeks, significantly ($p<0.05$) increased mean absolute weight of kidneys (112% of control). At 104 weeks, significantly ($p<0.05$) reduced mean body weight (52% of control), significantly increased ($p<0.05$) mean organ-to-body weight ratios of heart (115%) and adrenals (142%) compared with control.	
	F/60/group	93-148 g/ 5-6 weeks			0		30% mortality, exclusive of interim kill.	
					0.2		50% mortality, exclusive of interim kill.	
					0.68		30% mortality, exclusive of interim kill.	
					2.0		60% mortality, exclusive of interim kill. At 52 weeks, increased severity of interstitial nephritis and cytomegaly in epithelial cells of renal tubules in 6/10 rats, considered to be a marginal effect.	
Mouse/HaM/IcR	M/50/group	28 g/NR	NR	diet	0	78 weeks	40% mortality; clusters of nodules in stomach (nonglandular mucosa) in 0/28	Hazelton Laboratories, 1978
					0.28		41% mortality; stomach nodules (as above) in 13/26	
					0.91		30% mortality; stomach nodules in 24/28	
					-2.7		40% mortality; stomach nodules in 26/26; significantly ($p<0.05$) decreased hematocrit, hemoglobin content and RBC count.	
	F/50/group	22 g/NR	NR	diet	0		26% mortality; stomach nodules in 1/32	
					0.28		8% mortality, which was significantly ($p<0.05$) higher than control; stomach nodules in 10/44	
					0.91		27% mortality; stomach nodules in 26/34	
					2.7		20% mortality; stomach nodules in 38/38	

TABLE V-4 (cont.)

Species/Strain	Sex/No.	Weight/Age	Purity	Vehicle	Dose[a,b] (mg/kg bw/day)	Duration	Effects	Reference
Mouse/B6C3F$_1$	M/20 untreated 20 vehicle control	NR/5 weeks	>90% with 16 minor impurities	corn oil	0	78 weeks observation	No difference in mean body weight, hunched or thin appearance and deaths in high-dose from week 38. Significantly dose-related increased mortality ($p<0.001$). By week 47, 80% high-dose and by week 59, 84%, low-dose died. Vehicle control survival - 90% at week 58. By week 77, 80% untreated control died. Incidence of toxic tubular nephropathy 45/48 high-dose, 11/46 low-dose, 0/40 control.	NCI, 1977
					0 (vehicle)	59 weeks 0 weeks observation		
	50 treated per group				corrected TWA 81.4	60 weeks treated		
					correct TWA 156	47 weeks treated		
	F/20 untreated 20 vehicle controls	NR/5 weeks			0	90 weeks observation	No difference in mean body weight; hunched or thin appearance and deaths in high-dose from week 38. Significant dose-related increased mortality ($p<0.001$). By week 47, 61% high-dose and by 60, 82% low-dose died. Vehicle control survival - 90% at week 58. At 90 weeks, 95% untreated control survived. Incidence of toxic tubular nephropathy 43/46 high-dose, 14/50 low-dose, 0/40 control.	
					0 (vehicle)	60 weeks 0 weeks observation		
	50 treated per group				corrected TWA 78.6	60 weeks treated		
					corrected TWA 149.3	47 weeks treated		

[a] In the NCI (1977) studies, DBCP was administered 5 times/week at experimental TWA doses of 15 and 29 mg/kg bw for male and female rats, and 114 and 219 mg/kg bw for male mice and 110 and 209 mg/kg bw for female mice. The corrected TWA doses were calculated from the equation:

Corrected TWA Dose = Experimental TWA dose x (5 days ÷ 7 days) (see Tables V-5 and V-6).

[b] For the Dow-Hazleton studies (Hazleton Laboratories America, Inc., 1977, 1978), the nominal doses were 0.3, 1.0 and 3.0 mg/kg bw/day. The Carcinogen Assessment Group estimated that, after volatilization, the animals ingested the doses appearing in the table (U.S. EPA, 1979a; 44 FR 65135).

NR = Not reported; M = male; F = female

the 200, 100, 50 and 5 ppm DBCP groups, respectively, indicates cumulative DBCP intake during the study was proportional to its level in water. Using water consumption data and body weight data in the study report, daily DBCP consumption is estimated to be 9.38, 5.21, 3.17 and 0.37 mg/kg bw by the 200, 100, 50 and 5 ppm DBCP groups, respectively.

Body weight gain was lower ($p<0.05$) in the 200 ppm group. Significant ($p<0.05$) increases in OCT levels in serum were generally dose-dependent in the 5, 50 and 100 ppm groups, but there was no similar increase in the 200 ppm group except at final sacrifice. The authors concluded that the increased OCT levels were minimal evidence of liver injury, but there was no treatment-related effect on serum levels of GPT, GOT, BUN and SDH, which are other markers for cellular injury. No effects of DBCP treatment on organ weights, serum gonadotropin levels, epididymal sperm counts and testicular testosterone levels were found. Histopathological findings were unremarkable except for a slight increase in the number of nuclei in renal proximal tubules in rats given 100 and 200 ppm DBCP.

The authors stated that 64 days of ingestion of DBCP in the drinking water at 200, 100, 50 or 5 ppm had no significant effect on the reproductive system or liver or kidney function in the male Sprague-Dawley rat. However, they reported an additional experiment in which groups of four rats were given tap water alone or 100 or 200 ppm DBCP in drinking water for 5 days and glucose and protein levels in urine and the specific gravity of urine were increased ($p<0.05$) in both treated groups. The authors concluded that, although increased urinary specific gravity probably reflects decreased water consumption, increases in urinary glucose and protein reflect kidney injury. However, effects of treatment with 5 or 50 ppm DBCP on these urinary measurements were not assessed. A NOAEL is therefore not evident in this study.

In a preliminary subchronic study conducted to determine dose levels of DBCP to be administered to Osborne-Mendel rats and B6C3F$_1$ mice for carcinogenicity testing (NCI, 1977), doses of 100-631 mg/kg bw/day and 25-160 mg/kg bw/day were administered by gavage 5 days/week for 6 weeks to mice and rats, respectively. The doses represented in Table V-3 were calculated by multiplying the experimental doses by 5 days ÷ 7 days. The only effects of DBCP administration reported in the subchronic study were mortality data and changes in body weight. For mice, mean body weight gain was unaffected. A dose of $\geq$284 mg/kg bw/day resulted in 100% mortality. One male and one female died at unspecified lower doses. In rats, mean body weight gain was appreciably reduced in both sexes at 18 mg/kg bw/day. No deaths occurred at $\leq$29 mg/kg bw/day. Rats appear to be more sensitive to DBCP exposure than mice. The dose-response of DBCP administered in the diet to Long-Evans rats for 90 days was reported by Torkelson et al. (1961). Kodama and Dunlap (1956) also reported results of the study in abstract form. The concentrations of DBCP in the diet were 5, 20, 50, 150, 450 or 1350 mg/kg diet. Assuming that young growing rats consume an amount of food equal to 10% of their body weight per day (Mitruka et al., 1976), the corresponding doses are 0.5, 2.0, 5.0, 15.0, 45.0 and 135 mg/kg bw/day. The no-effect level in this study was 0.5 mg/kg bw/day. At $\geq$2.0 mg/kg bw/day female rats had significantly increased kidney-to-body weight ratios. This effect occurred in males only at 135 mg/kg bw/day. No effects besides the increased kidney weight were observed at 5 mg/kg bw/day. At 15.0 mg/kg bw/day, mean body weight gain of female rats was significantly reduced. This dose-effect is in good agreement with the dose of 18 mg/kg bw/day dose that produced reduced body weight gain in rats in the NCI (1977) subchronic gavage study. At the $\geq$45.0 mg/kg bw/day dose level, male rats had reduced mean body weight gain and females had increased liver-to-body weight ratios. Clinical signs of toxicity occurred at the two highest dose levels.

Rats had ruffled fur at 45.0 mg/kg bw/day and lethargy and muscular weakness at 135.0 mg/kg bw/day. Mortality at 135.0 mg/kg bw/day was 2/14 females and 4/14 males. Autopsy of the dead animals revealed discoloration of livers, kidneys and stomachs and edema of intestines.

Several studies of subchronic oral exposure to DBCP in rats were reported in the Russian literature (Rakhmatullayev, 1969, 1971; Faydysh et al., 1970; Reznik and Sprinchan, 1975).

Reznik and Sprinchan (1975) reported reduced spermatogenesis in male rats and atypical estrus cycles in female rats administered "multiple" oral doses (schedule unspecified) of 10 mg/kg bw for 4-5 months. These results are not incompatible with those reported for inhalation exposures (see Inhalation Exposure Section under Effects of Subchronic and Chronic Exposure). Histopathologic effects were described for daily oral doses of 70 mg/kg bw/day for 45 days, including hepatocellular necrosis and regeneration, bile duct proliferation, testicular necrosis and regeneration necrosis in the spleen and cytomegaly of epithelial cells of the nephron (Rakhmatullayev, 1969; Faydysh et al., 1970). Changes in hematologic and clinical blood chemistry parameters and reduced phagocytic activity of neutrophils were observed at this dose. Similar results were obtained when the 70 mg/kg/day dose was administered for 2.5 months. At lower doses (17.5 and 35 mg/kg bw/day) the reduced phagocytic activity of neutrophils was observed (Rakhmatullayev, 1971). Decreased phagocytic activity of neutrophils and effects on conditioned reflexes and generative function of gonads were reported at a dose as low as 0.05 mg/kg bw/day administered for 8 months (Rakhmatullayev, 1971). At 0.5 and 5 mg/kg bw/day, effects on hematologic parameters, increased BUN, proteinurea and effects on the testes were reported. As indicated in Table V-3, the effects seen at 0.05 mg/kg bw/day were not well defined and are of questionable physiologic significance and the dose may represent a no-observed-adverse-effect level.

Johnston et al. (1981) conducted a one-generation study with DBCP in rats. The DBCP sample was 97.3% pure (w/w) and contained 0.81% epichlorohydrin and 0.43% allyl chloride. Fresh solutions of DBCP in drinking water were made twice each week. The DBCP solutions were analyzed for concentration, stability and homogenicity by electron capture chromatography. Male and female Sprague-Dawley rats, initially 60 days old, were randomly selected for each dose group by body weight. Ten males and 10 females were assigned to groups given 0 (drinking water controls), 0.02, 0.2, 2.0 and 20.0 mg DBCP/kg bw/day. The animals consumed DBCP for 60 days before mating. Males were bred with two similarly treated females for a period of 5 days with one followed by the same period with the other. Treatment of males continued during the mating periods and females were treated during mating, gestation and the first 5 days of lactation. Adult males were killed 2 days after mating, and adult females were killed 3-5 days after the final parturition. Pups were killed 5 days after birth.

Observations were made for food and water intake; body weights; fertility, gestation and viability indices; litter size; pup survival; pup sex; litter weights; neonatal abnormalities; number of uterine implantation sites; and gross and histopathologic examinations of pups and adults. Major organs of adults were weighed.

Analysis of DBCP solutions indicated remarkable variability in the actual levels of DBCP, particularly at the high dose, primarily because of the difficulty in solubilizing DBCP. However, a dose-related consumption of DBCP was evident, and the DBCP intake observed with the three lowest doses was roughly equivalent to the desired intake.

There were no deaths and no clinical signs of toxicity. Food and water consumptions were markedly reduced in high dose males and females; however, DBCP levels in the drinking water given to the high dose group were increased accordingly to maintain the nominal dose. There was a significant ($p<0.05$) body weight loss in high dose males and females. High dose males showed significant ($p<0.05$) decreases in absolute heart, kidney and accessory sex gland weights and significant ($p<0.05$) increases in relative (to body weights) heart and adrenal weights. High dose females had significantly ($p<0.05$) decreased relative heart and adrenal weights and significantly ($p<0.05$) increased stomach weights. Average pup weights in the high dose group were significantly ($p<0.05$) lower on days 1 and 4 following birth. A discernable but not significant ($p<0.05$) decrease in the viability index was evident in the high dose group. No treatment-related pathology was found.

A NOEL of 2.0 mg/kg/day for systemic toxicity and reproductive effects is apparent in this study. This NOEL of 2.0 mg/kg/day corresponds to the estimated 3.17 mg/kg/day (50 ppm) dose in the Heindel et al. (1983) study where no treatment-related effects were found for the measured endpoints. However, kidney effects as increases in urinary outputs of glucose and protein and in urinary specific gravity were found with estimated exposure levels of 5.21 and 9.38 mg/kg/day (100 and 200 ppm) in the Heindel et al. (1983) study. These urinary measurements were made neither in the Johnston et al. study nor for the 50 and 5 ppm groups in the Heindel et al. (1983) study. The dose of 0.5 mg/kg/day in the Torkelson et al. (1961) study where DBCP was given in the diet to rats is roughly equivalent to the 0.2 mg/kg/day level in the Johnston et al. study and the estimated 0.37 mg/kg/day level in the Heindel et al. (1983) study. These doses are considerably below the effective estimated 5.21 mg/kg/day (100 ppm) dose in the Heindel et al. (1983) study and although without the actual data it is not certain, therefore probably do represent a NOEL for the effects on kidney function observed in the Heindel et al. (1983) study. However, without the specific data, there is a question about the 2 mg/kg/day dose in the Johnston et al. study and the estimated 3.17 mg/kg/day (50 ppm) dose in the Heindel et al. (1983) study actually being NOELs or NOAELs. These doses could possibly be effective in that they are rather close (~50%) to the lowest dose (estimated as 5.21 mg/kg/day) where remarkable effects on kidney function were observed in the Heindel et al. (1983) study.

Chronic Studies. Studies on the effects of chronic oral exposure of animals to DBCP are limited to studies designed to assess the carcinogenicity of the chemical (see Carcinogenicity Section). Nevertheless, some information on other toxic effects were presented along with data on tumor incidence. In the NCI (1977) bioassay, Osborne-Mendel rats and $B6C3F_1$ mice were treated by gavage with DBCP (>90% pure). The design summary and dosing schedules are presented in Tables V-5 and V-6, respectively, and the results are given in Table V-4. In rats, mean body weight gain was decreased in a dose-related manner. A high rate of mortality was observed among treated groups. High incidences of toxic tubular nephropathy, characterized by degeneration of proximal tubules, cloudy swelling, fatty degeneration and necrotic epithelia were observed in all treated rat groups: 49/50 high-dose males, 50/50 low-dose males, 44/44 high-dose females and 42/42 low-dose females. This lesion was not observed among control rats. Carcinomas of the forestomach in male and female treated rats and mammary tumors in treated females were treatment-related. The carcinogenic effects are discussed more fully in the Oral Exposure Section.

In an unpublished study conducted for the Dow Chemical Company (Hazelton Laboratories, 1977; U.S. EPA, 1979a; 44 FR 65135), groups of 60 male and 60 female Charles River rats were treated with DBCP in the diet for 104 weeks. The nominal doses were 0.3, 1.0 and 3.0 mg/kg bw/day. The Carcinogen Assessment Group (CAG) of the U.S. EPA estimated that, because of evaporation from the feed, the actual doses were 0.20, 0.68 and 2.0 mg/kg bw/day. Groups of 60 rats/sex that did not receive DBCP in the diet served as controls (U.S. EPA, 1979a). An interim kill of 10 rats/sex/group was performed at 52 weeks. There were no observed treatment-related effects of DBCP on

TABLE V-5

Design Summary for NCI Bioassay of DBCP in Osborne-Mendel Rats: Gavage Study[a]

Sex and Treatment Group	Initial No. of Rats[b]	DBCP Dose[c] (mg/kg bw)	Time on Study: Treated (weeks)	Time on Study: Untreated (weeks)	TWA Dose[d]	Corrected TWA Dose[e] (mg/kg bw/day)
MALE						
Untreated control	20	0	0	109	0	0
Vehicle control	20	0	78	5	0	0
Low-Dose	50	12	9		15	10.7
		15	69			
		0		5		
High-Dose	50	24	9	0	29	20.7
		30	55			
FEMALE						
Untreated control	20	0	0	109	0	0
Vehicle control	20	0	78	5	0	0
Low-Dose	50	12	9	0	15	10.7
		15	64			
High-Dose	50	24	9	0	29	20.7
		30	55			

[a] Source: NCI, 1977

[b] Treated and vehicle control rats were 7 weeks of age, untreated controls were 6 weeks of age when placed on test.

[c] The dose was administered by gavage on 5 consecutive days per week.

[d] TWA Dose = Σ(Dose x number of weeks received) ÷ Σ(weeks receiving treatment).

[e] Corrected TWA Dose = TWA Dose x $\frac{5 \text{ days}}{7 \text{ days}}$, calculated to expand the dose over a 7-day week.

TABLE V-6

Design Summary for NCI Bioassay of DBCP in B6C3F$_1$ Mice: Gavage Study[a]

Sex and Treatment Group	Initial No. of Mice[b]	DBCP Dose[c] (mg/kg bw)	Time on Study		TWA Dose[d]	Corrected TWA Dose[e] (mg/kg bw/day)
			Treated (weeks)	Untreated (weeks)		
MALE						
Untreated control	20	0	0	78	0	0
Vehicle control	20	0	59	0	0	0
Low-Dose	50	80 100 130	11 14 35	0	114	81.4
High-Dose	50	160 200 260	11 14 22	0	219	156.4
FEMALE						
Untreated control	20	0	0	90	0	0
Vehicle control	20	0	60	0	0	0
Low-Dose	50	60 100 130	11 14 35	0	110	78.6
High-Dose	50	120 200 260	11 14 22	0	209	149.3

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[a] Source: NCI, 1977

[b] Treated and vehicle control mice were 5 weeks of age when started on test. The age of the untreated controls was not reported, but it is assumed that they were approximately 5 weeks of age.

[c] The dose was administered by gavage on 5 consecutive days per week.

[d] TWA Dose = Σ(dose x number of weeks received) ÷ Σ(weeks receiving treatment).

[e] Corrected TWA Dose = TWA Dose x $\frac{5\text{ days}}{7\text{ days}}$, calculated to expand the dose over a 7-day week.

behavior or other outward signs of toxicity. Hematologic parameters, clinical chemistry values and results of urinalysis were not different among treated groups. Although there were no statistically significant differences in mortality at 104 weeks, somewhat higher mortality was observed in high-dose male and female groups, low-dose female group and medium-dose male group when compared with controls. Mean body weight of only the high-dose male group was statistically significantly reduced (52% of control; $p<0.05$) at 104 weeks. At necropsy, organ weights of heart, liver, spleen, kidneys, testes, epididymis, thyroid and adrenals were obtained. The high-dose male group had significantly increased mean absolute kidney weight (112% of control, $p<0.05$) at the interim sacrifice, and significantly ($p<0.05$) increased organ-to-body weight ratios of heart (115%) and adrenals (142%) compared with control at the 104-week terminal kill. Gross pathological and extensive histological examination of tissues of the control and high-dose interim kill groups were performed at 52 weeks. The liver, kidney and unusual lesions were examined histologically for the 10 rats/sex of the low- and medium-dose groups at the interim kill. There was no evidence of a carcinogenic effect of DBCP by 52 weeks. The only observed treatment-related histopathologic effect was an increased severity of interstitial nephritis in high-dose females accompanied by cytomegaly of epithelial cells in the renal tubules of 6/10 high-dose females. The incidence of renal tubule epithelial cytomegaly was not reported for other groups. Mortality of the rats by 52 weeks was very low with ≤ 2 deaths/group. Carcinogenic effects of DBCP observed in this study are discussed in the Oral Exposure Section under Carcinogenicity.

For mice, early mortality among treated male and female groups was observed. Mean body weight gain was unaffected by treatment. Toxic tubular nephropathy was observed in all treated groups with the following incidences: 45/48 high-dose males, 11/46 low-dose males, 43/46 high-dose females, and 14/50 low-dose females. This lesion was not observed in control mice. Carcinomas of the forestomach in treated groups were observed as discussed in the Oral Exposure Section under Carcinogenicity.

In an unpublished study conducted for Dow Chemical Company (Hazelton Laboratories, 1978; U.S. EPA, 1979a; 44 FR 65135), groups of 50 male and 50 female HaM/IcR Swiss mice were treated with DBCP in the diet for 78 weeks. The nominal doses were 0.3, 1.0 and 3.0 mg/kg bw/day. The CAG estimated that the mice had actually ingested 0.28, 0.91 and 2.7 mg/kg bw/day (U.S. EPA, 1979a). Groups of 50/sex that did not receive DBCP in the diet served as controls. There were no observable effects of DBCP feeding on behavior or other outward signs of toxicity during the 78-week period. Mean body weight gains and food consumption were similar among all groups. Information on mortality is presented in Table V-4. There were no treatment-related effects on clinical chemistry values or results of urinalysis. The only observed differences in hematological parameters were statistically significant ($p<0.05$) decrease in hematocrit, hemoglobin concentration and RBC counts in the high-dose male group compared with controls. All mice that died spontaneously, were killed when moribund, or were killed at the termination of the study, were necropsied. A dose-related increase in the incidences of clusters of white nodules in the nonglandular mucosa of the stomach was observed in male and female groups (see Table V-4). The kidneys, liver, stomach, testes, palpable tissue masses and suspected tumors were examined histologically in the high-dose and control groups. The incidences of tumors are presented in the Oral Exposure Section under Carcinogenicity. Papillomas and carcinomas were diagnosed in the stomachs of high-dose mice. In addition, acanthosis, hyperkeratosis and increased activity of basal cells were also observed.

Inhalation Exposure --

Subchronic Studies. The effects of subchronic inhalation exposure to DBCP have been studied in a variety of species. The details of the studies discussed in this section are presented in Table V-7.

Commonly observed effects of subchronic inhalation exposure were testicular atrophy and reduced spermatogenesis in male animals (Torkelson et al., 1961; Rao et al., 1982, 1983). Histopathologic damage to the upper respiratory tract, especially the nasal cavity, was observed in rats and mice (Torkelson et al., 1961; NTP, 1982a, Reznik et al., 1980a). Reduced body weight gain is a frequent observation after inhalation exposure (Kodama and Dunlap, 1956; Torkelson et al., 1961; NTP, 1982a; Rao et al., 1983), and mortality increases at high exposure levels (Torkelson et al., 1961).

In an early study, Kodama and Dunlap (1956) reported that inhalation of 5-50 ppm (48-483 mg/m^3) DBCP, delivered as 50 exposures over 10 weeks to rats, resulted in reduced body weight gain compared with controls. At 483 mg/m^3 DBCP, 3/15 rats died, but no effects on internal organs were observed at any concentration. More recently, dose-related effects, starting at concentrations as low as 1 ppm (9.7 mg/m^3) DBCP have been observed in laboratory animals (Rao, 1982, 1983; Torkelson et al., 1961; NTP, 1982a).

TABLE V-7

Effects of Subchronic Inhalation Exposure of Laboratory Animals to DBCP

Species/Strain	Sex/No.	Weight/Age	Purity	Concentration[a] (mg/m³)	Duration and Exposure Schedule	Effects	Reference
Rat/Long-Evans	M/15/group	NR/NR	NR	0, 48, 97, 193 or 483	10 weeks A total of 50 exposures, the duration of individual exposures NR	Reduced body weight gain was observed in all treated groups except the 97 mg/m³ group. At autopsy, no damage to organs and no changes in organ-to-body weight ratios were observed; 3/15 high-dose rats died.	Kodama and Dunlap, 1956
Rat/NR	M/15/group	NR/NR	95-98%	0	10 weeks Exposures were 7 hours/day 5 days/week	No observations were reported for the control group.	
				48		Decreased body weight gain and increased liver-to-body weight ratio compared with controls ($p<0.005$). At autopsy, there were focal changes in the epithelia of testes, renal tubules and bronchioles.	Torkelson et al., 1961
				97		2 deaths in 10 weeks; decreased body weight gain, increased liver and kidney-to-body weight ratios compared with controls ($p<0.05$). Rats had loss of hair and corneal dulling. At autopsy, there were pathologic changes in lungs, intestinal mucosa, kidneys and testes.	
				193		10 deaths in 7-10 weeks; decreased body weight gain, increased liver and kidney-to-body weight ratios compared with controls ($p<0.05$). Rats had corneal dulling and hair loss at 5 weeks. At autopsy, there were lesions of lungs, intestinal mucosa, kidneys and testes. Testes were atrophied. Lung collapse, emphysema and bronchopneumonia were observed.	
				387		13/15 died during the first 3 weeks. The surviving 2 rats were emaciated, had nasal discharge and died during week 5. Extensive testicular and renal changes were observed.	

TABLE V-7 (cont.)

Species/Strain	Sex/No.	Weight/Age	Purity	Concentration[a] (mg/m³)	Duration and Exposure Schedule	Effects	Reference
Rat/NR	M+F/20	NR/NR	95-98%	0 and 116	13 weeks Exposures were 7 hours/day 5 days/week	Body weight gain was significantly depressed in treated males and females. Mortality was 50% for males, 40% for females and was attributed to pulmonary infection. Effects on liver consisted of centrilobular congestion and sinusoid dilation in treated males and females. Treated females had increased kidney and lung-to-body weight ratios. Treated males had degeneration of seminiferous tubules; increased numbers of Sertoli cells, reduced sperm count and abnormal sperm. Treated males also had cloudy swelling of renal tubule epithelial cells.	Torkelson et al., 1961
Rat/F344	M/5/group	153.2 g/ 5-6 weeks	96% DBCP, 0.7% allyl chloride 0.8% epichlorohydrin 2.5% residue		13 weeks Exposures were 6 hours/day 5 days/week	No histological abnormalities of nasal cavities or other abnormalities in controls.	NTP, 1982a; Reznik et al., 1980a,b
		139.8 g/ 5-6 weeks		9.7		There was a 40% decreased mean body weight gain relative to controls; hepatocellular hydropic swelling; nephrosis; in nasal cavity, 1 had cytomegaly of basal cells, 2 had focal hyperplasia, 1 had squamous metaplasia, 3 had loss of cilia.	
		101.6 g/ 5-6 weeks		48		There was a 7% decreased mean body weight gain relative to controls; hepatocellular hydropic swelling, focal hepatic necrosis; nephrosis; in nasal cavity, 3 had cytomegaly of basal cell, 3 had focal hyperplasia, 2 had squamous metaplasia, 4 had loss of cilia.	

TABLE V-7 (cont.)

Species/Strain	Sex/No.	Weight/Age	Purity	Concentration[a] (mg/m^3)	Duration and Exposure Schedule	Effects	Reference
Rat/F344 (cont.)		137.2 g/ 5-6 weeks		242		One was killed when moribund. There was a 100% decreased mean body weight gain relative to controls (-0.4 g net body weight gain), bleeding from nose and mouth throughout the exposure, and severe hair loss. One rat had meningoencephalitis; 3 rats had vacuolated or necrotic adrenals; 5 had focal liver necrosis but with evidence of regeneration; 5 had renal tubular nephrosis and megalocytosis, 3 had tracheal and bronchial epithelial necrosis, squamous metaplasia of mucosa with evidence of regeneration and hyperplasia of bronchial epithelium; 3 had testicular atrophy and hypospermatogenesis; 5 had reduced bone marrow cell counts; 4 had atrophied thymus and lymphoid depletion; in the nasal cavity 4 had cytomegaly of basal cells, 5 had focal hyperplasia, 2 had squamous metaplasia, and 5 had loss of cilia. There was severe necrosis, inflammation and hemorrhage of respiratory system and nasal epithelium.	NTP, 1982a; Reznik et al., 1980a,b
Rat/F344	F/5/group	115.0 g/ 5-6 weeks		0	13 weeks Exposures were 6 hours/day 5 day/week	No nasal cavity or other histologic abnormalities.	NTP, 1982a; Reznik et al., 1980a,b
		118.6 g/ 5-6 weeks		9.7		There was a 5% decreased body weight gain relative to controls and nephrosis in the nasal cavity, 2 had basal cytomegaly, 2 had focal hyperplasia and 1 had loss of cilia.	
		105.8 g/ 5-6 weeks		48		There was a 12% increased body weight gain relative to controls; nephrosis in the nasal cavity, 4 had basal cell cytomegaly, 3 had focal hyperplasia, 1 had squamous metaplasia and 4 had loss of cilia.	

TABLE V-7 (cont.)

Species/Strain	Sex/No.	Weight/Age	Purity	Concentration[a] (mg/m³)	Duration and Exposure Schedule	Effects	Reference
Rat/F344 (cont.)		115.4 g/ 5-6 weeks		242		2 died, 2 were killed when moribund, 114% decreased body weight gain relative to control; severe hair loss; 4 had meningoencephalitis, 3 had vacuolated or necrotic adrenals, 5 had focal liver necrosis with regeneration, 5 had tubular nephrosis and cytomegaly, 3 had tracheal and bronchial epithelial necrosis, squamous metaplasia of mucosa with regeneration and hyperplasia of bronchial epithelia.	NTP, 1982a; Reznik et al., 1980a,b
Rat/Sprague-Dawley	F/30/group	225-300 g/ NR	97.3%	0, 0.97, 9.7 and 97	14 weeks followed by 26 weeks observation Exposures were 6 hours/day, 5 days/week	Interim kills of 5 rats/group at 4 and 14 weeks of exposure. No effects on mortality, clinical chemistry or hematology. Mean body weight of high-exposure group was slightly reduced compared with controls. No effect on fertility (see Teratogenicity and Other Reproductive Effects Section). No effects on organ weights. No histopathologic lesions were observed at 4-week kill. At 14 weeks there was focal cortical hyperplasia in adrenal glands of 3/5 at 97 mg/m³ exposure. At 26 weeks after exposure, the incidence of ovarian cysts was 7/20 in 97 mg/m³ group, 0/20 in the 9.7 mg/m³ group, 1/20 in 0.97 mg/m³ group and 1/20 in the control group. The incidence of nodular hyperplasia of the adrenal cortex was 18/20, 7/20, 0/20 and 0/20 in the 97, 9.7, 0.97 and 0 mg/m³ recovery groups, respectively.	Rao et al., 1983

TABLE V-7 (cont.)

Species/Strain	Sex/No.	Weight/Age	Purity	Concentration[a] (mg/m^3)	Duration and Exposure Schedule	Effects	Reference
Rat/Sprague-Dawley	M/30/group	350-450 g/ NR	97.3%	0, 0.97, 9.7 and 9.7	14 weeks followed by 32 weeks observation Exposures were 6 hours/day, 5 days/week	Interim kills of 5 rats/group at 4 and 14 weeks of exposure. No effect on mortality, no changes in clinical chemistry or hematologic parameters. Mean body weight of highest exposure group was slightly reduced compared with controls. No effect on fertility (see Teratogenicity and Other Reproductive Effects Section). There was an increased incidence of resorbed fetuses in females mated to high exposure males (see Mutagenicity Section) at <9.7 mg/m^3, no differences in brain, heart, liver and kidney weights at 4 or at 14 weeks. At 97 mg/m^3, testes and epididymis-to-body weight ratios were decreased and kidney-to-body weight ratio was increased at 14 weeks. Only testicular weight remained low at 32 weeks postexposure. No histopathologic damage to organs other than testes were observed at 4 weeks. Rats at 9.7 and 97 mg/m^3 levels had focal and multifocal atrophy of testes, decreased spermatogenesis and loss of spermatogenic elements. Sertoli cells had predominated in a few tubules at 4 weeks and at 14 weeks. At the 14-week kill, adrenal glands had focal cortical hyperplasia in 3/5 rats at 97 mg/m^3. At 32 weeks after exposure, 12/18 high-exposure rats had treatment-related, slight atrophy of testes. The other groups had low incidences of testicular atrophy that were believed to be age-related. Nodular hyperplasia of adrenal cortex was observed in 19/20 rats at 97; 1/20 at 9.7, 0/20 in 0.97 and 0/20 in 0 mg/m^3 exposure levels at the 32-week sacrifice.	Rao et al., 1983

TABLE V-7 (cont.)

Species/Strain	Sex/No.	Weight/Age	Purity	Concentration[a] (mg/m³)	Duration and Exposure Schedule	Effects	Reference
Mouse/B6C3F$_1$	M/10[b]	16.4 g/ 4-5 weeks	96% DBCP	0	13 weeks Exposures were 6 hours/day 5 days/week	No nasal cavity or other abnormalities.	NTP, 1982a Reznik et al., 1980a,b
	M/9	25.5 g	0.7% allyl chloride, 0.8% epichlorohydrin, 2.5% heavy ends	9.7		There was a 52% decrease in mean body weight gain relative to controls; 2 mice had occasional cytomegaly in bronchiolar epithelium. Nasal cavity: 2 had cytomegaly, 2 had focal hyperplasia, 2 had squamous metaplasia and 2 had loss of cilia.	
	M/8	16.9 g/ 4-5 weeks		48.3		There was an 11% decrease in mean body weight gain relative to controls, regeneration, hyperplasia and cytomegaly of bronchiolar epithelium. Nasal cavity: 5 had cytomegaly, 4 had focal hyperplasia, 3 had squamous metaplasia, and 3 had loss of cilia.	
	M/10	21.7 g/ 4-5 weeks		241.6		4 mice died. There was a 69% decrease in mean body weight gain relative to controls; hydropic changes in hepatocytes; nephrosis; necrosis of bronchiolar epithelium. Nasal cavity effects: 6 mice had cytomegaly of basal cells, 8 had focal hyperplasia, 5 had squamous metaplasia and 6 had loss of cilia. There was severe necrosis, inflammation and hemorrhage of respiratory and nasal epithelium.	
	F/10	16.6 g/ 4-5 weeks		0	13 weeks Exposures were 6 hours/day, 5 days/week	No nasal cavity or other abnormalities.	
	F/10	221 g/ 4-5 weeks		9.7		There was a 75% decrease in mean body weight gain relative to controls. Nasal cavity morphology: 3 mice had cytomegaly, 1 had focal hyperplasia, 1 had squamous metaplasia and 2 had loss of cilia.	
	F/12	17.3 g		48.3		1 mouse died. There was a 15% decrease in mean body weight gain relative to controls; regeneration, hyperplasia and cytomegaly of bronchiolar epithelium. Nasal cavity changes: 5 had cytomegaly, 5 had focal hyperplasia, 3 had squamous metaplasia and 4 had loss of cilia.	

TABLE V-7 (cont.)

Species/Strain	Sex/No.	Weight/Age	Purity	Concentration[a] (mg/m³)	Duration and Exposure Schedule	Effects	Reference
Mouse/B6C3F1 (cont.)	F/10	18.2 g/ 4-5 weeks		241.6		There was a 19% decrease in mean body weight gain relative to controls and necrosis of bronchiolar epithelium. Nasal cavity morphology: 7 mice had cytomegaly of basal cells, 7 had focal hyperplasia, 4 had squamous metaplasia and 4 had loss of cilia. There was severe necrosis, inflammation and hemorrhage of respiratory and nasal cavity epithelium.	NTP, 1982a Reznik et al., 1980c,d
Rabbit/New Zealand	M/10/group	NR/6 months	97.3%	0	46 weeks observation	At 14 weeks, 5% of the control sperm were abnormal.	Rao et al., 1982
				0.97	14 weeks, followed by 32 weeks observation	Interim kill of 3 or 4 rabbits at 14 weeks. No changes in clinical chemistry, hematology or mean body weight. No changes in testes weight. At 14 weeks, 10% of the sperm were abnormal.	
				9.7	14 weeks, followed by 32 weeks observation	Interim kill of 3 or 4 rabbits at 14 weeks. One rabbit died with rhinitis and pneumonia during exposure period. Sperm counts were significantly reduced ($p<0.05$) during 11-14 weeks of exposure with recovery observed at 10 weeks postexposure. There was no change in clinical chemisty, hematology or mean body weight. FSH levels were significantly increased at 14 weeks, but normal at 32 weeks postexposure. Testes weights were reduced to 50% of control at the 14-week kill and remained markedly reduced at 32 weeks postexposure. At 14 weeks, there were 18% abnormal sperm. At 32 weeks postexposure, there were 24% abnormal sperm.	
				97	8 weeks, followed by 38 weeks observation	One rabbit was killed at 8 weeks; 2 rabbits died during exposure period and had pneumonia. There were significantly reduced sperm counts during weeks 7 and 8 of exposure and they remained reduced throughout the observation period. The percentage of live sperm was significantly decreased from 8 weeks throughout observation period. No changes in clinical chemistry, hematology or mean body weight were observed.	

TABLE V-7 (cont.)

Species/Strain	Sex/No.	Weight/Age	Purity	Concentration[a] (mg/m³)	Duration and Exposure Schedule	Effects	Reference
Rabbit/New Zealand (cont.)				97	Exposures were 6 hours/day 5 days/week	There was 100% infertility (no female mated to exposed males 6 weeks after exposure became pregnant, (see Teratogenicity and Other Reproductive Effects Section). Fertility was restored in 2/5 exposed males mated at 33 weeks postexposure. Serum FSH levels were significantly elevated at 38 weeks postexposure. Testes weights were 25% of control at 8 weeks and were markedly reduced at 28 weeks postexposure. There was severe testicular atrophy with complete loss of spermatogenesis as early as 8 weeks. Only Sertoli cells were present. There was atrophy of epididymis, prostate and accessory genitalia. At 8 weeks, rabbits were aspermatozoic at 38 weeks after exposure; 20% of the sperm were abnormal.	Rao et al., 1982
Rabbit/NR	M+F/3/sex/group	NR/NR	95-98%	0 and 116	13 weeks Exposures were 7 hours/day 5 days/week	Males had significantly ($p<0.05$) decreased testes-to-body weight ratio compared with control. Males and females had slightly reduced body weight compared with controls. No other abnormalities were observed.	Torkelson et al., 1961
Guinea pig/NR	M+F/10/sex/group	NR/NR	95-98%	0 and 116	13 weeks Exposures were 7 hours/day 5 days/week	Testes-to-body weight ratio was significantly reduced in treated males compared with controls ($p<0.05$). Cloudy swelling and fatty changes in the liver was observed, but it was not reported whether this occurred in males or females or both.	Torkelson et al., 1961
Monkey/NR	F/2	NR/NR	95-98%	116	10 and 11 weeks Exposure was 7 hours/day 5 days/week	Both monkeys were moribund and emaciated, had severe secondary infections and aplastic anemia (believed to be a result of infection rather than DBCP exposure).	Torkelson et al., 1961

[a] Concentrations expressed as mg/m³ were calculated from exposures expressed as ppm in air:

$$\text{mg/m}^3 = \frac{\text{ppm} \times \text{M.W.}(236.3)}{24.45}$$

[b] The numbers of mice per group are as presented in NTP (1982). In Reznik et al. (1980c,d), the number of animals was reported to be 10 per group.

NR = Not reported; M = male; F = female

Torkelson et al. (1961) reported dose-related effects at 5-40 ppm (48-387 mg/m³) DBCP administered 7 hours/day, 5 days/week to male rats. At ≥48 mg/m³ DBCP, there was decreased body weight gain and increased liver-to-body weight ratio compared with controls. Focal histological changes were observed in testes, renal tubules and bronchioles. As the inhaled concentration increased, deaths occurred with increased incidence, kidney-to-body weight ratio was increased, histopathologic changes were observed with greater severity, testes became atrophied and lungs collapsed. Clinical signs included hair loss, corneal dulling, emaciation and nasal discharge. At the highest concentration, all of 15 rats were dead within 15 weeks; damage to testes and kidneys was extensive. In another study reported by Torkelson et al. (1961), the effects on male and female rats of exposure to 12 ppm (116 mg/m³) DBCP, 7 hours/day, 5 days/week for 13 weeks included decreased body weight, increased kidney and lung-to-body weight ratios. In addition, liver damage, consisting of centrilobular congestion and sinusoid dilation, was observed. Testicular damage was more fully described and included degeneration of seminiferous tubules, increased numbers of Sertoli cells, reduced sperm counts and abnormal sperm.

Rao et al. (1983) described dose-response data for testicular effects of inhaled DBCP in rats. Dose-related histopathologic effects on the adrenal cortex were also observed. A concentration of 0.1 ppm (0.97 mg/m³) DBCP, 6 hours/day, 5 days/week for 14 weeks had no effect on mortality, clinical chemistry, hematology, body weight, organ-to-body weight ratios, histopathology or testicular function. At 1 ppm (9.7 mg/m³) DBCP, there was focal necrosis and multifocal atrophy of testes, decreased spermatogenesis and loss of spermatogenic elements with Sertoli cells predominating. One of

20 rats examined had nodular hyperplasia of the adrenal cortex after 32 weeks of recovery. At 10 ppm (97 mg/m³), mean body weight was slightly reduced, testes and epididymis-to-body weight ratios were decreased and kidney-to-body weight ratios were increased. In the testes, there was focal and multifocal atrophy and reduced spermatogenesis. Focal hyperplasia of the adrenal cortex was observed in 3/5 rats at the 14-week kill. Nodular hyperplasia of the adrenal cortex was observed in 19/20 rats at the end of the 32-week recovery period. Testicular atrophy, unrelated to aging effects, was still observed after 32 weeks of recovery. Effects of 97 mg/m³ DBCP in female rats killed at 14 weeks were focal hyperplasia of the adrenal cortex in 3/5 rats and slightly reduced mean body weight. The incidence of nodular hyperplasia of the adrenal cortex increased in a dose-related manner. The incidence of ovarian cysts also appeared to be dose-related.

A preliminary subchronic inhalation study of 13 weeks duration was conducted in male and female rats to determine the concentrations of DBCP to be administered for carcinogenicity testing (NTP, 1982a). In a separate report of the same study, Reznik et al. (1980a) described the effects on the nasal cavity. Effects at 1 ppm (9.7 mg/m³) DBCP included reduced mean body weight gain, hepatocellular swelling (males), nephrotic and histologic changes in the nasal cavity. At 5 ppm (48 mg/m³) DBCP the reduced body weight in males was less pronounced than at 9.7 mg/m³; females gained weight relative to controls at 48 mg/m³. It should be noted that the initial weights of the rats were quite variable, and the data may not be a good indicator of body weight changes. The effects on liver and kidney became more severe and nasal cavity lesions were observed with greater frequency. At 25 ppm (242 mg/m³) DBCP, rats were emaciated. Clinical signs included nasal bleeding and severe hair loss. Extensive histopathologic damage including necrosis of adrenals, liver, renal tubules, tracheal and bronchial epithelia and testes, was observed. Evidence of regeneration was observed in damaged tissues. Bone marrow cell counts were reduced, thymus glands were atrophied and lymphoid cells were depleted. Nasal cavity lesions included severe necrosis, inflammation and hemorrhage. Several rats (1 male, 4 females) had meningoencephalitis.

A preliminary subchronic inhalation study was also conducted in male and female mice to determine exposure levels for carcinogenicity testing (NTP, 1982a); nasal cavity histopathology was described by Reznik et al. (1980a). The concentrations of DBCP and exposure schedules were the same as in the rat study and similar results were obtained. Mean body weight gain reduction was observed at all exposure levels but did not follow a dose-related pattern. Again, the initial weights of the mice were quite variable. At 9.7 mg/m³ DBCP, there was an occasional cytomegalic bronchiolar epithelial cell; at 48 mg/m³ DBCP, the bronchiolar epithelium was hyperplastic; at 242 mg/m³ DBCP, the bronchiolar epithelium was necrotic. Nasal cavity lesions occurred with dose-related increased incidences and severity. Mice appear to be less sensitive to DBCP inhalation than rats since no effects on adrenals, bone marrow, thymus or kidneys were observed. However, species sensitivity is often determined by potency in one organ. Hence the number of organs affected may be irrelevant. Mice appear to be less sensitive than rats but this is not necessarily so.

In rabbits, effects of DBCP inhalation on body weight and testicular morphology and function have been observed (Torkelson et al., 1961; Rao et al., 1982). The effects on spermatogenesis were partially reversible after exposure ceased (Rao et al., 1982). As in rats, a concentration of 0.1 ppm (0.97 mg/m³) DBCP, 6 hours/day, 5 days/week, resulted in no effect on body weight, clinical chemistry, hematology or testes weights in rabbits. At this concentration, however, there were 10±3% abnormal sperm compared with 5±3% abnormal sperm in control rabbits. At 1 ppm (9.7 mg/m³) DBCP, sperm counts were reduced, but recovery was observed after 10 weeks postexposure. Testicular weight was reduced and remained reduced throughout the observation period. The percentage of abnormal sperm had increased from 18% at the end of exposure to 24% by the end of 32 weeks postexposure. Rabbits that had been exposed to 10 ppm (97 mg/m³) DBCP were infertile as evidenced by 0% pregnancy in bred females, and complete loss of spermatogenesis at 8 weeks of exposure. Fertility was restored in 2/5 rabbits mated 33 weeks after exposure was terminated as evidenced by the ability to impregnate female rabbits. As observed by microscopic examination of testicular tissue, some spermatogenic capacity was restored, but there were 20% abnormal sperm.

Reduced testes-to-body weight ratio was observed in guinea pigs exposed to 12 ppm (116 mg/m³) DBCP 7 hours/day, 5 days/week for 13 weeks. Female monkeys, similarly exposed, were emaciated and moribund (Torkelson et al., 1961).

Chronic Studies. In the NTP (1982a) bioassay of carcinogenicity of inhaled DBCP in F344 rats and B6C3F$_1$ mice (see Inhalation Exposure Section under Carcinogenicity), data on toxic effects other than tumor incidences were also reported. The details of the study are presented in Table V-8.

In male and female rats exposed to 0, 0.6 or 3.0 ppm (0, 5.8 and 29 mg/m^3) DBCP for 6 hours/day, 5 days/week for $\geq$84 weeks, dose-related effects on kidney, stomach and nasal cavity histology were observed. Toxic tubular nephropathy (cytomegalic nuclei and focal hyperplasia in tubule cells) occurred in 94% of high-dose females, 100% of high-dose males and 4% of the low-dose males. This condition was not observed in low-dose females or controls of either sex. A dose-related increased incidence of hyperkeratosis of the esophagus was observed in male rats. This lesion was not found in female rats. The incidence of stomach lesions, consisting of chronic inflammation, acanthosis and hyperkeratosis, increased as the concentration of DBCP increased in both sexes. Nasal cavity inflammation, hyperplasia and hyperkeratosis were observed in all treated groups, but not in controls. Mean body weight gain reduction and increased mortality occurred in high-dose groups.

Dose-related effects on kidney, stomach and nasal cavity histology were also observed in mice similarly exposed to DBCP vapor (NTP, 1982a). Toxic tubular nephropathy was not found in female mice, nor in control male mice. The incidence of toxic tubular nephropathy was lower in low-dose male mice (2%) and high-dose male mice (20%) than in low-dose male rats (4%) and high-dose male rats (100%). Nasal cavity and other upper respiratory tract lesions were not seen in control mice, but the incidences increased with

TABLE V-8

Effects of Chronic Inhalation Exposure of Rats and Mice to DBCP[a]

Species/Strain	Sex/No.	Weight/ Age	Purity	Concentration[b] (mg/m^3)	Duration and Exposure Schedule	Effects
Rat/F344	M/50	NR/5-6 weeks	96% DBCP 0.7% alkyl chloride, 0.8% epichlorohydrin 2.5% residue	0	105-107 weeks observation	41/50 survived 104-107 weeks; 0/50 had toxic tubular nephropathy. The incidence of hyperkeratosis of esophagus was 1/48, of acanthosis of stomach was 1/50, of chronic inflammation of the stomach was 1/50 and of hyperkeratosis was 0/50.
	M/50	NR/5-6 weeks		5.8	103 weeks, followed by 1 week observation	42/50 survived 104-107 weeks; 2/50 had toxic tubular nephropathy; 4/47 had hyperkeratosis of esophagus; 1/49 had chronic inflammation of the stomach; 1/49 had hyperkeratosis and 1/49 had acanthosis of stomach. Nasal cavity inflammation and hyperplasia were observed with increased frequency over control.
	M/49	NR/5-6 weeks		29	84 weeks, followed by 1 week observation	Decreased mean body weight gain (22%) beginning at week 65. There was significantly increased mortality compared with low-dose and control groups ($p<0.001$). At 84 weeks, 5/49 were alive. Toxic tubular nephropathy was observed in 49/49 rats. The incidences of stomach lesions were: hyperkeratosis, 9/49; acanthosis, 6/49; chronic inflammation, 5/49. Hyperkeratosis of esophagus was observed in 18/49 rats. Inflammation of the nasal cavity and hyperplasia occurred with increased frequency over controls.
	F/50	NR/5-6 weeks		0	105-107 weeks observation	42/50 survived 104-107 weeks. No toxic tubular nephropathy was observed, 1/50 had inflammations, 2/50 had hyperkeratosis, and none had acanthosis of stomach.
	F/50	NR/5-6 weeks		5.8	103 weeks, 1 week observation	42/50 survived 104 weeks. Nasal cavity hyperplasia (48%), abscess and hyperkeratosis was observed in this group. Incidence of stomach lesions: inflammation, 2/50; hyperkeratosis, 2/5; acanthosis, 3/50. No toxic tubular nephropathy was observed.

TABLE V-8 (cont.)

Species/Strain	Sex/No.	Weight/ Age	Purity	Concentration[b] (mg/m^3)	Duration and Exposure Schedule	Effects
Rat/F344 (cont.)	F/50	NR/5-6 weeks		29	84 weeks, 0-1 week observation all exposures 6 hours/day 5 days/week	Decreased mean body weight gain (12%) beginning at week 65. Increased mortality compared with control and low-dose group ($p<0.001$). 6/50 were alive at 84 weeks. Nasal cavity hyperplasia (46%) abscess, hyperkeratosis and squamous metaplasia observed frequently. Stomach lesion incidences: inflammation, 7/48; hyperkeratosis, 15/48; acanthosis, 12/48. Toxic tubular nephropathy observed in 46/47 (94%) rats of this group.
Mouse/B6C3F$_1$	M/50/ group	NR/4-5 weeks	96% DBCP 0.7% alkyl chloride 0.8% epi-chlorohydrin 2.5% heavy ends	0	80 weeks observation	13/50 survived 76 weeks; no nasal or respiratory lesions; no stomach lesions; no tubular nephropathy.
				5.8	76 weeks, followed by 0-1 of observation	15/50 survived 76 weeks. Incidences of nasal cavity and other respiratory lesions: hemorrhage, 1/42; focal hyperplasia, 2/42; focal hyperplasia of bronchiole, 7/20. Incidence of stomach lesions: focal necrosis, 1/41; hyperkeratosis, 10/41; acanthosis, 6/41. Incidence of toxic tubular nephropathy, 1/42.
				29	76 weeks followed by 0-1 observations	After 60 weeks, mean body weight gain was decreased 17-18%. 7/50 survived 76 weeks (35% survived 70 weeks). Incidence of nasal cavity lesions: hemorrhage, 4/48; serous inflammation, 5/48; suppurative inflammation, 21/48; focal hyperplasia, 12/48. Incidence of other respiratory lesions: focal hyperplasia of larynx, 5/48; of trachea, 4/41; of bronchus, 14/45; of bronchiole, 29/45 and of alveolor epithelium, 7/45. Incidence of stomach lesions: hemorrhage, 3/44; chronic inflammation, 2/44; hyperkeratosis, 17/44; acanthosis, 11/44. Incidence of toxic tubular nephropathy, 9/46.

TABLE V-8 (cont.)

Species/Strain	Sex/No.	Weight/ Age	Purity	Concentration[b] (mg/m³)	Duration and Exposure Schedule	Effects
Mouse/B6C3F$_1$ (cont.)	F/50/ group	NR/4-5 weeks		0	104 weeks observation	40/50 survived 104 weeks; no nasal cavity or other respiratory lesions; no stomach lesions; no toxic tubular nephropathy.
				5.8	103 weeks, 1 week observation	37/50 survived 104 weeks. Incidences of nasal cavity and other respiratory lesions: serous inflammation, 4/50; suppurative inflammation, 5/50; focal hyperplasia, 17/50; focal hyperplasia of larynx, 3/50; hyperplasia (NOS) of bronchiole, 5/49; hyperplasia of alveolar epithelium, 5/49. Incidence of stomach lesions: chronic inflammation, 2/48; hyperkeratosis, 20/48; acanthosis, 12/48. Tubular nephropathy was not observed in female mice. Spleen atrophy was observed in 3/48 mice.
				29	76 weeks, followed by 0-1 week observation Exposures were 6 hours/day 5 days/week	After 60 weeks, mean body weight gain was depressed 25%; 43/50 died between week 51 and 74. Incidence of respiratory lesions: serous inflammation of nasal cavity, 4/50; suppurative inflammation of nasal cavity, 13/50; focal hyperplasia, 3/50; hyperplasia of larynx (NOS), 3/50; hyperplasia of bronchiole (NOS), 11/47; hyperplasia of alveolar epithelium, 11/47. Incidences of stomach lesions chronic inflammation, 4/46; focal necrosis, 3/46; hyperkeratosis, 24/46; acanthosis, 18/46. Spleen atrophy was observed in 19/46 mice. Tubular nephropathy was not observed in female mice.

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[a] Source: Adapted from NTP, 1982a

[b] Concentrations expressed as mg/m³ were calculated from concentrations expressed as pm in air:

$$\text{mg/m}^3 = \frac{\text{ppm x M.W.(236.3)}}{24.45}$$

NR = Not reported; NOS = not otherwise specified; M = male; F = female increasing DBCP concentration in both male and female treated groups. The incidence of stomach lesions, also not observed in control mice, increased in a dose-related manner in male and female treated mice.

Target Organ Toxicity

Effects on Reproductive Organs. Histopathologic changes in the testes were observed in several species following acute and subchronic oral and inhalation exposures to DBCP. Acute oral doses of ≥100 mg/kg bw in rats resulted in degeneration of seminiferous tubules, testicular atrophy, degeneration of caput epididymal epithelium, decreased sperm counts and decreased sperm motility (Saito-Suzuki et al., 1982; Reznik and Sprinchan, 1975). Testicular necrosis with regeneration was observed in rats after 45 days of oral doses of 70 mg DBCP/kg bw/day (Rakhmatullayev, 1969; Faydysh et al., 1970). Similar effects were observed after subchronic inhalation exposures in rats (NTP, 1982a). Damage to testes progressed from decreased number of germ cells and minor atrophy of some seminiferous tubules to severe necrosis, abnormal sperm morphology and loss of spermatogenic elements as inhalation concentrations and duration increased (Torkelson et al., 1961; Saeguso et al., 1982). Decreased weights of testes, epididymis, prostate and accessory genitalia among rats, rabbits and guinea pigs have been reported following inhalation exposures (Torkelson et al., 1961; Rao et al., 1982, 1983). Reduced spermatogenesis was reversible in rabbits after DBCP exposure ceased (Rao et al., 1982). Rao et al. (1983) also found a high incidence of ovarian cysts in DBCP-exposed female rats. Altered estrus cycles have been reported for female rats following oral exposures (Reznik and Sprinchan, 1975).

Several reports have described biochemical changes in the testes of rats. Testicular delta-aminolevulinic acid synthetase activity was increased, and testicular microsomal heme and cytochrome P-450 content was depressed in rats following oral exposure (Moody et al., 1982a; Tofilon et al., 1980). Increased activities of aryl hydrocarbon hydroxylase and epoxide hydrolase in testes of rats (Suzuki and Lee, 1981), and reduced content of testicular reduced glutathione (GSH) of mouse and rat testes have been reported (Kluwe et al., 1981).

Effects on Kidney. Following oral exposures of DBCP in rats, kidney weight was increased and proximal tubule cells were necrotic (Kato et al., 1980; Kluwe, 1981a). Toxic tubular nephropathy (degeneration of proximal tubules, cloudy swelling, fatty degeneration and necrosis) was observed in rats and mice after chronic oral exposure (NCI, 1977), and subchronic and chronic inhalation exposure (NTP, 1982a). Cytomegalic tubule epithelial cells were also observed after inhalation exposure (Saegusa et al., 1982; NTP, 1982a) and after oral exposure (Faydysh et al., 1970) in rats. Kidney damage became more severe as inhalation concentrations increased (Torkelson et al., 1961).

Dose-related increased severity of kidney damage was observed in rats after subcutaneous dosing with up to 120 mg/kg bw (Kluwe, 1981a). The changes were associated with increased urinary excretion of glucose, protein, GOT, LDH, N-acetyl-beta-D-glucosamine, alkaline phosphatase, gamma-glutamyl transpeptidase and Na^+ and K^+. BUN and serum creatinine levels were also increased. Other biochemical changes in kidneys associated with DBCP exposure included increased aryl hydrocarbon hydroxylase and epoxide hydrolase activities in rats (Suzuki and Lee, 1981), decreased microsomal cytochrome P-450 content in rats (Moody et al., 1982a) and decreased GSH content of kidneys of rats and mice (Kluwe et al., 1981).

Effects on Liver. Effects of DBCP exposure on the liver include mild hepatocellular swelling progressing to necrosis in rats with increasing acute subcutaneous doses up to 120 mg/kg bw (Kluwe, 1981a). Focal necrosis was observed following an acute oral lethal dose to rats (Kato et al., 1980). Subchronic oral doses produced similar hepatic changes with evidence of regeneration and bile duct proliferation (Faydysh et al., 1970). Regeneration of necrotic livers and hydropic swelling were also observed after subchronic inhalation in rats (NTP, 1982a). Torkelson et al. (1961) found centrilobular congestion and sinusoid dilation in rats and cloudy swelling and fatty changes in livers of guinea pigs after subchronic inhalation exposure to DBCP.

Biochemical changes in the liver of rats include increased aryl hydrocarbon hydroxylase and epoxide hydrolase activities (Suzuki and Lee, 1981); increased palmitic and linoleic acid content and decreased stearic and arachidonic acid content (Moody et al., 1980); increased liver protein and RNA content (Moody et al., 1982b); decreased microsomal cytochrome P-450 content (Moody et al., 1982a) and decreased liver GSH content (Kluwe et al., 1981).

Effects on the Stomach and Esophagus. Hyperkeratosis of the esophagus and lesions of the stomach, such as hyperkeratosis, acanthosis, chronic inflammation and focal necrosis were observed with a dose-related increased severity and incidence in rats and mice after chronic gavage dosing with DBCP (NTP, 1982a). Carcinomas of the forestomach were also observed and are discussed in the Carcinogenicity Section.

Effects on Tissues and Organs of the Respiratory System. Following continuous inhalation exposure to DBCP for 14 days, rats had bronchial distention, emphysema, alveolar distention and cytomegalic and necrotic bronchiolar epithelial cells (Saegusa et al., 1982). Concentration-related changes from focal bronchiolar lesions to more severe necrosis occurred (Torkelson et al., 1961). Lung collapse and emphysema were also observed.

Lesions of the upper respiratory tract, particularly the nasal cavity, became more severe and occurred with increased incidence in rats and mice as inhalation concentrations of DBCP increased (NTP, 1982a; Reznik et al., 1980a). Nasal cavity lesions included cytomegaly of basal cells, focal hyperplasia, squamous metaplasia, loss of cilia, severe necrosis, inflammation and hemorrhage of nasal epithelium. Tracheal and bronchial epithelia were hyperplastic and necrotic. Mice had focal hyperplasia of the larynx, trachea, bronchus, bronchiole and alveolar epithelia. Nasal cavity carcinomas and adenomas were observed in mice and rats and are discussed in the Carcinogenicity Section.

Effects on Hematopoietic Organs and Blood Cells. Atrophy of white pulp and decreased numbers of lymphocytes in red pulp of the spleen was observed as a transient effect of inhalation exposure in rats (Saegusa et al., 1982). Atrophy of the spleen of mice occurred after chronic inhalation exposure (NTP, 1982a). Necrosis of red and white pulp was also noted after subchronic oral exposure in rats (Rakhmatullayev, 1969, 1971). Decreased hemoglobin content and decreased red and white blood cell counts were observed as well as decreased phagocytic activity of neutrophils (Rakhmatullayev, 1969, 1971). Reduced numbers of bone marrow cells, atrophied thymus gland and lymphoid depletion were observed in rats after subchronic inhalation exposures (NTP, 1982a).

Effects on CNS. CNS depression, apathy, sluggishness and ataxia were observed in rats after high acute inhalation exposures ($\geq$580 mg/m^3 for 1-7 hours) (Torkelson et al., 1961).

Effects on Adrenal Gland. There was focal hyperplasia of the adrenal cortex in male and female rats exposed to subchronic inhalation of DBCP (Rao et al., 1983).

Effects on Skin and Eyes. Slight erythema, crustiness, necrosis of dermis and subcutaneous tissue, hyperemia and scaliness were observed after dermal exposure of rabbits to DBCP. Slight pain and irritation of conjuctiva and iris occurred after DBCP was applied to the eyes of rabbits (Torkelson et al., 1961). Dulling of the cornea occurred in rats exposed to repeated inhalations of DBCP (Torkelson et al., 1961).

Other Effects

Studies on the carcinogenicity, mutagenicity, teratogenicity and reproductive effects of DBCP exposure in laboratory animals are discussed in this section.

Carcinogenicity. The carcinogenic effects of DBCP have been studied in rats and mice that had been exposed orally by gavage (NCI, 1977), orally by dietary administration (Hazelton Laboratories, 1977, 1978; 44 FR 65135), by inhalation of DBCP vapors in air (NTP, 1982a) and dermally in mice (Van Duuren et al., 1979). Several separate published reports that were based upon data of the NCI gavage bioassay (Olson et al., 1973; Powers et al., 1975; Weisburger, 1977) and the NTP inhalation bioassay (Reznik et al., 1980b,c,d) are available but provide little additional pertinent information to that in the final NCI (1977) and NTP (1982a) reports.

Oral Exposure -- In the NCI (1977) bioassay of DBCP in rats, groups of 50 male and 50 female Osborne-Mendel rats were administered a corn oil solution of technical grade DBCP (>90% purity with ~16 minor contaminants that were not identified) orally by gastric intubation, 5 days/week for ≥64 weeks. Corrected TWA high- and low-doses, calculated by multiplying the experimental doses by 5 days ÷ 7 days, were 10.7 and 20.7 mg/kg bw/day, respectively. Controls consisted of 20 male and 20 female rats that received no treatment and 20 male and 20 female rats that were treated by gavage with corn oil. The design of the dose schedule and duration of experiment for the various groups (durations varied because of early mortality in some groups) were previously presented in Table V-5. Comprehensive histological examination was performed on major organs and gross lesions of all animals that died spontaneously, were killed when moribund, or were killed at the termination of study. Histological examination of some tissues was precluded in some cases because of partial autolysis. Mortality data and signs of toxicity were discussed in the Effects of Subchronic and Chronic Exposure Section. The incidences of treatment-related tumors are summarized in Table V-9. Highly significant increased incidences of squamous cell carcinoma of the forestomach, with extensive invasion of adjacent organs, were observed in all treated groups compared with no incidence of this tumor type in either control group ($p<0.001$ for all treated groups). The incidences for both male and female rats were dose-related ($p<0.001$), but with significant departure from linearity ($p<0.001$). The forestomach squamous cell carcinoma had metastasized to lungs in 5/50 low-dose males, 5/50 high-dose males and 3/44 high-dose females. A positive dose-response for the incidence of squamous cell papilloma of the forestomach in female rats was found ($p<0.003$), and the incidence in high-dose females was significantly different from either control group ($p=0.034$). The incidence of papillomas in males was not significant. A highly significant increased incidence of mammary adenocarcinoma was found for low- and high-dose females compared with either control group ($p<0.003$). The incidence was significantly dose-related ($p<0.001$) when determined using either control group.

The incidence of hemangiosarcoma in low-dose males was significantly different from either control group ($p=0.008$). This tumor did not occur in either high-dose group, but was observed in 4/42 low-dose females ($p>0.05$). It was suggested that because of a high rate of early deaths of high-dose rats, they may not have been at risk long enough.

TABLE V-9

The Incidence of Treatment-Related Tumors in Osborne-Mendel Rats Following Chronic Oral (Gavage) Exposure to DBCP[a,b,c]

	Squamous Cell Carcinoma of Forestomach		Squamous Cell Papilloma of Forestomach		Adenocarcinoma of Mammary Gland		Hemangiosarcoma of Spleen	
	Incidence	%	Incidence	%	Incidence	%	Incidence	%
			MALE					
Untreated control	0/20	0	0/20	0	1/20	5	0/20	0
Vehicle control	0/20	0	0/20	0	0/20	0	0/20	0
Low-dose	47/50	94	1/50	2	1/50	2	9/50	18
High-dose	47/50	94	2/50	4	0/50	0	0/49	0
			FEMALE					
Untreated control	0/20	0	0/20	0	2/20	10	0/20	0
Vehicle control	0/20	0	0/20	0	0/20	0	0/20	0
Low-dose	38/50	76	1/50	2	24/50	48	4/42	0
High-dose	29/49	59	9/49	18	31/50	62	0/44	0

[a] Source: NCI, 1977

[b] Tumor Incidence = $\frac{\text{Number of Tumor Bearing Rats}}{\text{Number of Animals Necropsied}}$ or $\frac{\text{Number of Tumor Bearing Rats}}{\text{Number of Tissues Examined}}$

[c] Results of statistical analysis are presented in text.

An unpublished chronic dietary bioassay of DBCP carcinogenicity in Charles River rats was performed by the Hazelton Laboratories (1977) for the Dow Chemical Company. This study has also been summarized by U.S. EPA (1979a; 44 FR 65135). The doses were expressed as mg/kg bw/day rather than as dietary concentrations in ppm or mg/kg diet. Each group consisted of 60 male and 60 female rats that were fed diets containing DBCP [DBCP was 95% pure and contained no epichlorohydrin but did contain $\leq$3.4% allyl chloride (44 FR 65135)] at nominal doses of 0.3, 1.0 or 3.0 mg/kg bw/day for 104 weeks. The CAG estimated that, after volatilization from the feed, the rats consumed doses of 0.20, 0.68 and 2.0 mg/kg bw/day (U.S. EPA, 1979a; 44 FR 65135). A group of 60 male and 60 female rats received food containing no DBCP. At week 52, an interim kill of 10 rats/sex/group was performed. The only histopathologic changes observed by 52 weeks were a dose-related slightly increased severity of interstitial nephritis and cytomegaly in the epithelium of renal tubules of high-dose females (2.0 mg/kg bw/day) (see Chronic Studies Section under Effects of Subchronic and Chronic Exposure). Surviving rats were killed at 104 weeks. The results, as tabulated by U.S. EPA (1979a), are presented in Table V-10. High-dose (2.0 mg/kg bw/day) male and female rats had significantly greater incidences of adenoma and carcinoma of the renal tubules and squamous cell carcinoma of the stomach than controls ($p<0.05$). The incidence of hepatocellular carcinoma was significantly increased over controls in high-dose males ($p<0.05$). When hepatocellular carcinoma and neoplastic nodules were considered together, significantly greater incidences of liver tumors were observed in low-dose (0.20 mg/kg bw/day) males, high-dose males and medium-dose (0.68 mg/kg bw/day) females.

TABLE V-10

Incidence of Tumors in Charles River Rats Treated with Dietary Levels of DBCP[a]

	Males				Females			
Dose (mg/kg bw/day)[b]	0	0.20	0.68	2.0	0	0.20	0.68	2.0
Number of rats examined	48	46	46	41	48	45	47	43
Renal tubules								
Adenoma	0	0	1	6[c]	0	0	0	7[c]
Carcinoma	0	1	3	9[c]	0	1	0	5[c]
Total	0	1	4	15[c]	0	1	0	12[c]
Stomach, squamous cell								
Papilloma	0	0	0	1	0	0	0	2
Carcinoma	0	0	3	20[c]	0	0	0	8[c]
Total	0	0	3	21[c]	0	0	0	10[c]
Liver								
Neoplastic nodules	0	4	2	3	0	2	2	3
Hepatocellular carcinoma	0	1	2	5[c]	0	1	3	0
Total	0	5[c]	4	8[c]	0	3	5[c]	3

[a] Source: U.S. EPA (1979a; 44 FR 65135), report of Hazelton Laboratories study for Dow Chemical Company

[b] Dose as estimated by CAG

[c] Significantly different from control ($p<0.05$)

The NCI (1977) bioassay of DBCP in $B6C3F_1$ mice was conducted similarly to the rat study. The numbers of animals were the same, but the corrected TWA doses (experimental TWA dose multiplied by 5 days ÷ 7 days) were 81.4 and 156.4 mg/kg bw/day for the low- and high-dose male mice, respectively, and 78.6 and 149.3 mg/kg bw/day for the low- and high-dose female mice, respectively. The design of the dose schedule and durations of exposure were presented in Table V-6. Comprehensive histological examinations was performed on major organs and gross lesions of animals that died spontaneously, were killed when moribund or were killed at the termination of the study. Histological examination was precluded in some animals that were lost, cannibalized or autolyzed. Histological examination of some tissues was not performed in some mice that died early. Mortality data and signs of toxicity were discussed previously in the Effects of Subchronic and Chronic Exposure Section. The only treatment-related tumor type observed in mice was squamous cell carcinoma of the forestomach. The incidence was as follows: 0/40 untreated controls, 0/40 vehicle controls, 43/46 (93%) low-dose males, 47/49 (96%) high-dose males, 50/50 (100%) low-dose females and 47/48 (98%) high-dose females. Statistical analysis indicated a positive dose-response ($p<0.001$) for both treated male and female groups compared with either respective control group. The incidence in all treated groups was significantly different from either respective control group ($p<0.001$). The squamous cell carcinoma of the forestomach had extensively invaded adjacent tissues. Metastases in lungs were observed in 4/46 low-dose males, 3/48 high-dose males, 10/50 low-dose females and 3/46 high-dose females.

Hazelton Laboratories (1978) conducted an unpublished chronic dietary bioassay of DBCP in HaM/IcR Swiss mice for the Dow Chemical Company. The results were also reported by U.S. EPA (1979a; 44 FR 65135). Doses were expressed as mg/kg/day rather than as dietary concentration expressed as ppm or mg/kg diet. Each group consisted of 50 male and 50 female mice that were fed diets containing DBCP (95% pure) at nominal doses of 0, 0.3, 1.0 and 3.0 mg/kg bw/day. Because of volatilization, CAG estimated that the mice actually received 0, 0.28, 0.91 and 2.7 mg/kg bw/day (U.S. EPA, 1979a). Gross pathological examination of all mice that died spontaneously, were killed when moribund, or killed at the end of the study, was performed. Histopathologic data for the high-dose group and the control group only were reported by Hazelton Laboratories. According to the Hazelton report, there was a dose-related increased incidence of nodules in the stomach in all treated groups (see Chronic Studies Section under Effects of Subchronic and Chronic Exposure). Treatment-related tumors consisted of squamous cell papillomas and carcinomas of the stomach. The incidences of papillomas were 6/49 high-dose males, 6/50 high-dose females and 0/100 male and female controls. The incidences of carcinomas were 26/49 high-dose males, 19/50 high-dose females and 0/100 male and female controls. Acanthosis, hyperkeratosis and increased activity of basal cells were also observed.

Inhalation Exposure -- In the NTP (1982a) bioassay of DBCP in rats, groups of 50 male and 50 female F344 rats were exposed to DBCP vapors in air in an inhalation chamber. Similar groups of control rats were exposed to filtered air. The DBCP used was ~96% pure (the preparation contained ~0.8% epichlorohydrin and 0.7% allyl chloride). Exposures were for 6 hours/day, 5 days/week for 84 (high-dose) or 103 (low-dose) weeks. The high-dose was 3 ppm (29 mg/m^3) and the low-dose was 0.6 ppm (5.8 mg/m^3). Comprehensive histological examination was performed on all major organs, tissues and gross lesions of all rats that died spontaneously, were killed when moribund or were killed at the termination of the study. Histological examination of some tissues was precluded in some cases because of partial autolysis. Mortality data and signs of toxicity were discussed in the Effects of Subchronic and Chronic Exposure Section. The incidences of treatment-related (and possible treatment-related) tumors are presented in Table V-11. The results of statistical analysis, where appropriate, are included in the table. Tumors considered to be treatment-related included all neoplasms of the nasal cavity, nasal turbinate, tongue, pharynx and larynx. Nasal cavity tumors included adenomatous polyps, adenomas, squamous-cell papillomas, squamous-cell carcinomas, carcinomas (not otherwise specified) and adenocarcinomas (not otherwise specified). Benign tumors were observed with greater incidences in low-dose rats, while malignant tumors were predominant in high-dose animals. Taken as a whole, the incidences of nasal cavity tumors in treated rats were highly significantly different from controls and followed a highly significant dose-related trend. Squamous-cell papillomas of the tongue followed a significant dose-related trend and were significantly higher in the high-dose groups than in controls. The incidences of squamous-cell carcinoma of the tongue followed dose-related trends but were not significantly different from controls. Very low incidences of tumors of the larynx in treated males and females and of the pharynx in treated male rats were observed, but were not seen among control rats. The incidence of squamous-cell papilloma of the pharynx in high-dose females was significantly different from controls. Tubular cell adenomas and adenocarcinomas of the kidney were observed with low incidence in treated males while none

TABLE V-11

Incidence of Treatment-Related and Possible Treatment-Related Tumors in F344 Rats Following Inhalation Exposure to DBCP^{a,b,c}

	Males			Females		
	Control	Low-Dose	High-Dose	Control	Low-Dose	High-dose
Number of Rats Examined	50	50	49	50	50	50
NASAL CAVITY						
Adenomatous polyp	0 (NS)	13 (p<0.001)	1 (NS)	0 (NS	5 (p=0.028)	5 (p=0.028)
Adenoma, NOS	0 (p<0.001)	9 (p=0.001)	1 (NS)	0 (NS)	6 (p=0.013)	5 (p=0.028)
Squamous-cell papilloma	0 (NS)	7 (p=0.006)	3 (NS)	0 (NS)	10 (p=0.001)	3 (NS)
Squamous-cell carcinoma	0 (p<0.001)	2 (NS)	11 (p<0.001)	1 (p=0.016)	2 (NS)	6 (NS)
Carcinoma, NOS	0 (p<0.001)	2 (NS)	22 (p<0.001)	0 (p<0.001)	0 (NS)	23 (p<0.001)
Adenocarcinoma, NOS	0 (NS)	8 (p=0.003)	6 (p=0.012)	0 (p=0.005)	2 (NS)	6 (p=0.013)
Number of rats with nasal cavity tumors	0 (p<0.001)	32 (p<0.001)	39 (p<0.001)	1 (p<0.001)	21 (p<0.001)	42 (p<0.001
NASAL TURBINATE						
Adenomatous polyp	0 (NS)	8 (p=0.003)	0 (NS)	0 (NS)	5 (p=0.028)	1 (NS)
Number of rats with nasal cavity and turbinate tumors	0 (p<0.001)	40 (p<0.001)	39 (p<0.001)	1 (p<0.001)	27 (p<0.001)	42 (p<0.001)
TONGUE						
Squamous-cell papilloma	0 (p<0.001)	1 (NS)	8 (p=0.003)	0 (p=0.011)	3 (NS)	6 (p=0.013)
Squamous-cell carcinoma	0 (p=0.010)	0 (NS)	3 (NS)	0 (p=0.040)	1 (NS)	3 (NS)
Numbers of rats with tongue tumors	0 (p<0.001)	1 (NS)	11 (p<0.001)	0 (p=0.002)	4 (NS)	9 (p=0.001)
LARYNX						
Squamous-cell papilloma	0	0	0	0	0	1
Sqbamous-cell carcinoma	0	1	0	0	0	0
Number of rats with larynx tumors	0	1	0	0	0	1

TABLE V-11 (cont.)

	Males			Females		
	Control	Low-Dose	High-Dose	Control	Low-Dose	High-dose
Number of Rats Examined	50	50	49	50	50	50
PHARYNX						
Squamous-cell papilloma	0	2	1	0 (p=0.001)	0 (NS)	5 (p=0.028)
Squamous-cell carcinoma	0	1	0	0 (NS)	0 (NS)	2 (NS)
Number of rats with pharnyx tumors	0	3	1	0 (p<0.001)	0 (NS)	6 (p=0.013)
KIDNEY						
Tubular-cell adenoma	0 (NS)	3 (NS)	1 (NS)	0	0	0
Tubular-cell adenocarcinoma	0 (NS)	1 (NS)	1 (NS)	0	0	0
Numbers of rats with kidney tumors	0 (NS)	4 (NS)	2 (NS)	0	0	0
MISCELLANEOUS						
Mammary fibroadenoma	0	0	0	4 (NS)	13 (p=0.016)	4 (NS)
Adenoma of adrenal cortex[d]	1 (NS)	6 (NS)	3 (NS)	0 (NS)	7 (p=0.006)	5 (p=0.025)
Mesothelioma of tunica vaginalis	0 (p=0.004)	1 (NS)	5 (p=0.027)	NA	NA	NA
Trichoepitheliomas of skin	0 (p=0.037)	1 (NS)	3 (NS)	0	0	0

[a] Source: NTP, 1982a

[b] The incidence of tumors expressed in this table is greater than the incidence of animals with tumors because more than one type of tumor was detected in some of the rats.

[c] p values for Fisher exact test, when significant, appear beside the incidence for treated rats; p values for dose-related trend, when significant, appear beside the incidence in controls. If the incidence is not significantly different from control or does not show a positive dose-related trend, NS (not significant) appears beside the incidence. Where NS or p values do not appear, statistical analyses were not performed.

[d] The number of male rats for which the adrenal cortex examined was 49, 49 and 48 for control, low-dose, and high-dose groups, respectively. For high-dose females, the number of rats examined for adrenal cortex lesions was 48.

NOS = Not otherwise specified; NA = not applicable were found in controls. These tumors were not observed in female rats, but they were considered to be treatment-related because they are unusual in F344 rats. Increased incidences of mammary fibroadenomas and of adenoma of the adrenal cortex in female rats, and of trichoepitheliomas of the skin and of mesotheliomas of the tunica vaginalis in male rats represented equivocal results since these tumors are commonly observed in F344 rats.

Table V-12 summarizes the results of a bioassay that was conducted similarly in B6C3F$_1$ mice (NTP, 1982a). Experimental details are identical to those in the rat bioassay except that the duration of the treatment period was 76-103 weeks (see Table V-8). Histological examination of some mice and tissues was precluded by cannibalism or autolysis. Data on mortality and signs of toxicity were presented in the Effects of Subchronic and Chronic Exposure Section.

Treatment-related tumors of the nasal cavity included adenomatous polyps, squamous-cell carcinomas, carcinomas (not otherwise specified) and adenocarcinomas (not otherwise specified), and were found with statistically significant increased incidences among high-dose males and females and showed dose-related trends when compared with controls. Statistically significant dose-related increases were also observed for carcinosarcoma and fibrosarcoma in high-dose females, although the incidences were not significantly different from controls. When all nasal cavity tumor-bearing mice/group were combined, there were highly significant dose-related trends for males and females and highly significant increased incidences in low-dose females and in high-dose males and females when compared with respective controls. The incidence of lung tumors were also related to DBCP exposure.

TABLE V-12

Incidence of Treatment-Related and Possible Treatment-Related Tumors in B6C3F$_1$ Mice Following Chronic Inhalation to DBCP^{a,b,c}

	Males			Females		
	Control	Low-Dose	High-Dose	Control	Low-Dose	High-Dose
Number of Mice Examined	45	42	48	50	50	50
NASAL CAVITY						
Adenomatous polyp	0 (p=0.007)	1 (NS)	5 (p=0.033)	0 (NS)	3 (NS)	0 (NS)
Squamous-cell papilloma	0	0	1	0	2	0
Squamous-cell carcinoma	0 (p=0.001)	0 (NS)	6 (p=0.016)	0 (p=0.002)	1 (NS)	6 (p=0.013)
Carcinoma, NOS	0 (p<0.001)	0 (NS)	7 (p=0.008)	0 (p<0.001)	4 (NS)	17 (p<0.001)
Adenocarcinoma, NOS	0	0	2	0 (p=0.005)	2 (NS)	6 (p=0.013)
Neoplasm, NOS, malignant	0	0	1	0	0	1
Carcinosarcoma	0	0	0	0 (p=0.011)	0 (NS)	3 (NS)
Fibrosarcoma	0	0	0	0 (p=0.011)	0 (NS)	3 (NS)
Sarcoma, NOS	0	0	0	0	0	1
Keranthoacanthoma	0	0	0	0	0	1
Hemangiosarcoma	0	0	3	0	0	0
Number of mice with nasal cavity tumors	0 (p<0.001)	1 (NS)	21 (p<0.001)	0 (p<0.001)	11 (p<0.001)	38 (p<0.001)
LUNG						
Alveolar/bronchiolar						
Adenoma	0 (p=0.003)	1 (NS)	6 (p=0.017)	3 (p=0.005)	3 (NS)	10 (p=0.029)
Carcinoma	0 (NS)	2 (NS)	1 (NS)	1 (NS)	2 (NS)	4 (NS)
Squamous-cell carcinoma	0	0	0	0	0	1
Squamous-cell carcinoma (bronchus)	0	0	1	0	0	1

TABLE V-12 (cont.)

	Males			Females		
	Control	Low-Dose	High-Dose	Control	Low-Dose	High-Dose
Number of Mice Examined	45	42	48	50	50	50
Papillary adenoma (bronchus/bronchiole)	0	0	0	0	0	1
Papillary carcinoma (Bronchus/Bronchiole)	0	0	3	0 (NS)	7 (p=0.006)	4 (NS)
Number of mice with lung tumors	0 (p<0.001)	3 (NS)	11 (p<0.001)	4 (p=0.001)	12 (p=0.027)	18 (p<0.001)
Number of mice examined	45	42	48	0	0	0
CIRCULATORY SYSTEM						
Hemangiosarcoma	0 (p=0.015)	0 (NS)	3 (NS)	0	0	0
STOMACH						
Squamous-cell papillomas	0 (p=0.045)	0 (NS)	2 (NS)	0	0	0
Squamous-cell carcinomas	0	0	1	0	0	0
Number of mice with stomach tumors	0 (p=0.016)	0 (NS)	3 (NS)	0	0	0

[a] Source: NTP, 1982a

[b] The incidence of tumors expressed in this table is greater than the incidence of mice with tumors becasue more than one type of tumor was detected in some of the mice.

[c] p values for Fisher exact test, when significant, appear beside tumor incidences in treated mice. p values for dose-related trend, when significant, appear beside tumor incidences in controls. If the incidence is not significantly different from control or does not show a positive dose-related trend, NS (not significant) appears beside the incidence.

NOS = Not otherwise specified

Statistically significant dose-related trends were observed for males and females when all lung-tumor-bearing mice/group were combined. Significantly increased incidences of lung tumors occurred in low-dose females and in high-dose males and females when compared with controls. Other tumors, possibly related to DBCP treatment, were hemangiosarcoma of the circulatory system, and squamous-cell papillomas and carconomas of the stomach in high-dose male mice.

As discussed in the report of the NTP (1982a) bioassays in rats and mice, the DBCP was contaminated with low levels of epichlorohydrin (0.8%) and 1,2-dibromoethane (0.07-0.7% of stored DBCP). Epichlorohydrin induced nasal cavity tumors in rats at an inhalation concentration of 100 ppm (970 mg/m^3 (Laskin et al., 1980); 1,2-dibromoethane induced nasal cavity tumors in rats and mice at inhalation concentrations of 10 and 40 ppm (97 and 388 mg/m^3) (NTP, 1982b). It was estimated that the maximum level of epichlorohydrin to which the animals had been exposed in the DBCP bioassays was 0.021 ppm (0.2 mg/m^3). The NTP (1982a) report, while stating that the contaminants did not likely cause the DBCP-induced nasal tumors, did not rule out a possible cocarcinogenic effect of the contaminants with DBCP.

Dermal Exposure -- Van Duuren et al (1979) tested DBCP as a tumor-initiator and as a whole carcinogen by dermal application in male and female HA:1 cr Swiss mice that were 6-8 weeks at the beginning of treatment. Treated groups consisted of 30 mice. The DBCP used was technical grade that had been purified by distillation. For the initiation-promotion test, a single dose of 69 mg DBCP/mouse was applied in 0.2 mℓ acetone to the dorsal skin. After a 2-week period, the tumor-promoter phorbol myristate acetate, was applied in a concentration of 5 mg/0.2 mℓ acetone 3 times/week for at least 60 weeks. The first tumor appeared at day 257. Papillomas were found in six mice with one papilloma/mouse. The tumor incidence of the control groups was not reported; however, the results of a chi-square analysis indicated that the incidence was significant ($p<0.05$). For the whole carcinogenicity bioassay, DBCP was applied to the shaved dorsal skin in doses of 11.7 and 35.0 mg/application/mouse in 0.2 mℓ acetone, 3 times/week for at least 60 weeks. In the high-dose group, one papilloma occurred in one mouse at day 326. No papillomas were induced in the low-dose group. Thus, DBCP was inactive as a whole carcinogen in mouse skin. The incidences of distant tumors, however, were highly significant. For the high-dose group, 23/30 mice had lung papillomas ($p<0.01$), 5/30 had carcinomas of forestomach and 10/30 had squamous-cell carcinomas of the forestomach ($p<0.0005$ for combined forestomach tumors). For the low-dose group, 29/30 mice had lung papillomas ($p<0.0005$), 15/30 had papillomas of the forestomach and 5/30 had squamous-cell carcinomas of the forestomach ($p<0.0005$ for combined forestomach tumors). The statistical analyses compared incidences in treated mice with incidences in control mice, but incidence data for controls were not reported.

Mutagenicity. DBCP has been tested for mutagenicity and related effects in a number of systems (Table V-13). In the reverse mutation assay with *Salmonella typhimurium*, positive results were obtained with tester strains TA1535, TA1530, TA100 and TA98 without metabolic activation (Prival et al., 1977; Rosenkranz, 1975; Moriya et al., 1983). Prival et al. (1977) reported that DBCP was not mutagenic in *S. typhimurium* strain TA1535 in the presence of S-9, while Moriya et al. (1983) found that the mutagenic

TABLE V-13

Mutagenicity Testing of DBCP

Assay	Indicator/ Organism	Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Reverse mutation	*Salmonella typhimurium* TA1535	NR	plate incorporation	0-1.0 μl/plate	+S-9 -S-9	- +	S-9 was prepared from Aroclor 1254 treated rats. Without S-9, the response was positive at ≥0.01 μl/plate. With S-9, DBCP increased the number of revertant colonies not quite 2-fold over controls at 0.01 μg/plate.	Prival et al., 1977
Reverse mutation	*S. typhimurium* TA1530 TA1538	NR	plate incorporation	0-20.9 mg/plate	-S-9 -S-9	+ -	+ at ≥0.4 mg/plate for TA1530.	Rosenkranz, 1975
Reverse mutation	*S. typhimurium* TA1535 TA100 TA1537 TA1538 TA98	NR	plate incorporation	0-5000 μg/plate	-S-9 -S-9 -S-9 -S-9 -S-9	+ + - - +	The response was + when the number of revertants was > control + 100 for TA100 or 3 x control for other strains. Also, a dose-response relationship was shown. The authors stated that the mutagenicity was enhanced by the addition of S-9. The dose-response for TA100 only was presented. With S-9, the response was greatly enhanced (~16-fold) over the response without S-9 at 1000 μg/plate.	Moriya et al., 1983
Reverse mutation	*S. typhimurium* TA1535	96% DBCP, 1.5% epichlorohydrin	plate incorporation	0-1600 μg/plate	-S-9	+	The response was weakly positive at ≥50 μg/plate.	Biles et al., 1978
Reverse mutation	*S. typhimurium* TA1535	99% DBCP, 0% epichlorohydrin	plate incorporation	0-1600 μg/plate	-S-9	-	No doubling of revertant rate over control was observed at any dose.	Biles et al., 1978
Reverse mutation	*S. typhimurium* TA1535	96% DBCP, 1.5% epichlorohydrin	plate incorporation	0-200 μg/plate	+S-9	+	S-9 was prepared from Aroclor treated rats. The response was strongly positive at ≥20 μg/plate.	Biles et al., 1978
Reverse mutation	*S. typhimurium* TA1535	99% DBCP, 0% epichlorohydrin	plate incorporation	0-200 μg/plate	+S-9	+	Same as above	Biles et al., 1978

TABLE V-13 (cont.)

Assay	Indicator/ Organism	Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Reverse mutation	*S. typhimurium* TA1535	96% DBCP, 1.5% epichlorohydrin	vapor phase	0-100 µℓ/plate	-S-9	+	The response was + at ≥1.0 µℓ/plate, i.e., more mutagenic than when tested in plate incorporation and more mutagenic than pure DBCP tested in vapor phase.	Biles et al., 1978
Reverse mutation	*S. typhimurium* TA1535	99% DBCP, 0% epichlorohydrin	vapor phase	0-100 µℓ/plate	-S-9	+	The response was + at ≥10 µℓ/plate.	Biles et al., 1978
Reverse mutation	*S. typhimurium* TA100	99.8% DBCP	plate incorporation	0-10 µmole/plate	+S-9 -S-9	+ -	S-9 was prepared from Aroclor 1254 treated rats. With S-9, the response was + at ≥0.25 µmole/plate. S-9 was absolutely necessary with the highly purified DBCP.	Stolzenberg and Hine, 1979
Reverse mutation	*S. typhimurium* TA98	99.8% DBCP	plate incorporation	0, 1 and 10 µmole/plate	+S-9 -S-9	+ -	A weakly positive response was obtained with 1 µmole/plate (1.7 x control) and with 10 µmole/plate (2.4 x control).	Stolzenberg and Hine, 1979
Reverse mutation	*Escherichia coli* WP2 hcr	NR	plate incorporation	0-5000 µg/plate	-S-9	+	The response was + when the number of revertant colonies was 3 x the control and there was a dose-relationship. The addition of S-9 was reported to enhance the mutagenicity.	Moriya et al., 1983
Rec- assay	*E. coli* pol A_1^- pol A^+	NR	spot test	20.9 mg/plate	-S-9	+	The growth of pol A_1^- was inhibited to a greater extent than was pol A^+.	Rosenkranz, 1975
Recessive lethal (Basc test)	*Drosophila melanogaster* progeny of SK males x Basc females	NR	exposure of males to DBCP vapors	0 and 20 µℓ soaked onto filter paper for 5 minutes	NA	+	15/622 lethal X-chromosomes in treated groups vs. 0/502 lethal X-chromosomes in control ($p<0.01$).	Inoue et al., 1982
Recessive lethal (Basc test)	*D. melanogaster* progeny of Canton-S males x Basc females	NR	exposure of males to DBCP vapors	0 and 60 ppm for 30 minutes	NA	-	No significant difference between treated groups and control in the number of recessive lethal mutations.	Kale and Baum, 1982a

TABLE V-13 (cont.)

Assay	Indicator/ Organism	Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Recessive lethal (Basc test)	D. melanogaster progeny of males x Basc females	NR	males treated as eggs to DBCP vapors	0 and 75 ppm for 30 minutes	NA	+	No significant increase over concurrent controls; highly significant increase over cumulative controls when not corrected for clusters. When the cluster was viewed as a single event, significant results were marginal ($p<0.10$).	Kale and Baum, 1982b
Recessive lethal (Basc test)	D. melanogaster progeny of Canton-S males x Basc females	NR	males fed on solution of DBCP in 0.01% ethanol	0 and 0.2 mg/mℓ for 72 hours	NA	+	143/1505 (9.5%) lethal x-chromosomes vs. 0/970 (0%) in ethanol controls. The results were considered positive although no statistical analysis was presented.	Zimmering, 1983
Heritable translocation	D. melanogaster progeny of Canton-S males x y:bw; stp^P females	NR	males fed on a solution of DBCP in 0.01% ethanol	0 and 0.2 mg/mℓ for 72 hours	NA	+	8/951 (0.8%) for heritable translocations vs. 0/946 (0%) in ethanol controls. The response was considered positive although no statistical analysis was presented.	Zimmering, 1983
Heritable translocation	D. melanogaster progeny of Canton-S males x bw;st females	NR	males treated with DBCP vapor	0 and 75 ppm for 30 minutes	NA	-	No significant difference between treated and control groups for absence of brown and scarlet eye color (indication of heritable translocation).	Kale and Baum, 1982b
Genetic crossing over	D. melanogaster progeny of vg/bw/++ heterozygous males x vg bw females	NR	males treated with DBCP vapor	0, 120 or 240 ppm for 30 minutes	NA	+	+ at higher exposure compared with controls.	Kale and Baum, 1982b
Chromosome loss	D. melanogaster progeny of $x^{C2}yf/B^{S}Y\underline{y}^{+}$ males x $y^{2}v$ or mei-9^{a} females	NR	males fed on solution of DBCP in 0.01% ethanol	0 and 0.2 mg/mℓ for 24 or 72 hours	NA	+	For 24-hour treatment: repair-proficient female ($y^{2}v$), 0.38% chromosome loss ($p=0.05$); repair-deficient female (mei-9^{a}), 0.75% loss ($p=0.05$). For 72-hour treatment: $y^{2}v$, 1.68% loss ($p=0.01$); mei-9^{a}, 3.55% loss ($p=0.01$).	Zimmering, 1983

TABLE V-13 (cont.)

Assay	Indicator/ Organism	Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
Sister chromatid exchange	Chinese hamster cells, V79	pure DBCP synthesized by authors	cell culture	1×10^{-5} to 1×10^{-3} M	NA	+	+ dose-related increased incidence of sister chromatid exchange/cell at $\geq1\times10^{-5}$ M ($p=0.01$ to 0.001).	Tezuka et al., 1980
Polyploid test	Chinese hamster cells, V79	pure DBCP synthesized by authors	cell culture	0 and 1×10^{-5} to 1×10^{-3} M	NA	-	No significant increase in polyploid cells/total cells observed when compared with controls.	Tezuka et al., 1980
Chromosome aberrations	male rats	NR	treated by gavage	0.73, 7.3 and 73 mg/kg bw/day for 5 days	NA	+	Dose-response increased incidence of aberrant cells in spermatogonia and bone marrow. Numerical data was not provided.	Kapp, 1979
Dominant lethal	Sprague-Dawley rat	NR	males treated by gavage	0, 10 or 50 mg/kg bw/day for 5 days	NA	+	Statistically significant increased incidence of dead implants were observed in the 50 mg/kg group at matings during weeks 1 through 5 post-treatment, and in the 10 mg/kg group in matings during weeks 4 and 5 as compared with controls.	Teramoto et al., 1980
Dominant lethal	Sprague-Dawley rat	>95%	males treated by gavage	0 and 50 mg/kg bw/day for 5 days	NA	+	Confirmation of previously reported results of Teramoto et al., 1980.	Saito-Suzuki et al., 1982
Dominant lethal	Sprague-Dawley rat	97.3%	males treated by inhalation	0, 0.97, 9.7 and 97 mg/m³, 6 hours/day, 5 days/week, 14 weeks	NA	+	Statistically significant increased incidence of resorbed fetuses in mated females compared with controls ($p<0.05$).	Rao et al., 1979, 1983
Dominant	BDF$_1$ mice	NR	males treated	0, 50 or 100	NA	-	Males were mated with 2 females/week for 6 consecutive weeks.	Teramoto et al., 1980
UDS	CD-1 mice (prepubertal)	>99%	males treated i.p.	100, 120 and 130 mg/kg bw	NA	+	Significant increase with time in UDS over hydroxyurea controls at all dose levels, $p<0.05$ at 8 hours after treatment.	Lee and Suzuki, 1979

TABLE V-13 (cont.)

Assay	Indicator/ Organism	Purity	Application	Concentration or Dose	Activating System	Response	Comment	Reference
UDS	CD-1 mice (adult)	>99%	males treated i.p.	80, 100 and 120 mg/kg bw	NA	-	No UDS was observed in mature spermatozoa.	Lee and Suzuki, 1979
Sperm morphology	(C57Bl/6XC3H)F_1 mice	technical grade	i.p.	5, 10, 19, 38, 75, 150 or 300 mg/kg bw/day for 5 days.	NA	-	No increased percent abnormal sperm at any dose compared with controls.	Osterloh et al., 1983

NR = Not reported; NA = not applicable

response in strains TA1535, TA100 and TA98 was enhanced in the presence of S-9. The mutagenicity of DBCP was also enhanced by metabolic activation in the reverse mutation assay and the rec assay in Escherichia coli (Moriya et al., 1983; Rosenkranz, 1975. Biles et al. (1978) also found that the mutagenic activity of DBCP (96% pure) in strain TA1535 of S. typhimurium was enhanced by metabolic activation. These investigators demonstrated that much of the direct mutagenicity of DBCP was actually due to contamination by epichlorohydrin. The preparation of DBCP in the study contained ~1.5% epichlorohydrin. When a highly purified preparation of DBCP (99% with no detectable epichlorohydrin) was assayed in strain TA1535, the response was negative without, but positive with, metabolic activation. Testing of epichlorohydrin itself in the S. typhimurium system resulted in a mutagenic response approximately the same as that induced by the impure DBCP preparation (data not included in Table V-13). Thus, while pure DBCP alone was not mutagenic with S-9, metabolic activation resulted in a positive response. Contamination of DBCP with epichlorohydrin resulted in a positive response, which was also enhanced by metabolic activation. Epichlorohydrin has been proposed as an intermediate in the metabolism of DBCP (Jones et al., 1979) and may thus be responsible for DBCP mutagenicity with activation. 2-Bromoacrolein, a potent mutagen (Rosen et al., 1980), has also been proposed as a metabolic intermediate of DBCP (Marsden and Casida, 1982) (see Metabolism Section in Chapter III). Biles et al. (1978) were unable to rule out the direct mutagenicity of pure DBCP in strain TA1535 of S. typhimurium since a weakly positive response was obtained when the assay was performed using the dessicator technique (vapor phase application). A highly purified preparation of DBCP (99.8%) absolutely required metabolic activation when assayed in strains TA100 and TA98 of S. typhimurium (Stolzenberg and Hine, 1979).

There was marginal activity, which was dose-related in TA98 at 1 and 10 μmol/plate. The positive response was dose-related at ≥0.25 μmole/plate in TA100. Epichlorohydrin, epibromohydrin, α-chlorohydrin and α-bromohydrin, proposed intermediates in DBCP metabolism (Jones et al., 1979; see Metabolism Section in Chapter III), were all positive without S-9 activation (Stolzenberg and Hine, 1979). Although all these compounds were also active in the presence of S-9, the activity was lower for epichlorohydrin and epibromophydrin. DBCP was not mutagenic in the reverse mutation assay in _S. typhimurium_ with strains TA1537 and TA1538 without metabolic activation (Rosenkranz 1975; Moriya et al., 1983).

DBCP has been studied for mutagenicity in the recessive lethal assay in _Drosophila melanogaster_ with positive (Inoue et al., 1982; Zimmering, 1983) and negative (Kale and Baum, 1982a) results. Kale and Baum (1982b) obtained a positive response for recessive lethal mutations when the male flies were treated as embryos. Testing of DBCP in _D. melanogaster_ for heritable translocations has also produced equivocal results with a positive response reported by Zimmering (1983) and a negative response reported by Kale and Baum (1982b). DBCP was positive for genetic crossing over (Kale and Baum, 1982b) and for chromosome loss (Zimmering, 1983) in _D. melanogaster_. The purity of DBCP in these studies was not specified. Dominant lethal assays were positive in rats (Teramoto et al., 1980; Saiko-Suzuki et al., 1982; Rao et al., 1979, 1983) but negative in mice (Teramoto et al., 1980). Other positive results include induction of sister chromatid exchange in cultured Chinese hamster V79 cells (Tezuka et al., 1980); chromosome aberrations in spermatogonia and bone marrow of rats treated with DBCP _in vivo_ (Kapp, 1979) and unscheduled DNA synthesis in germ cells of treated prepubertal mice (Lee and Suzuki, 1979). When adult mice were treated with DBCP, however, no unscheduled DNA synthesis was observed in mature spermatozoa (Lee and Suzuki, 1979). Other negative results were found in the polyploid test in Chinese hamster V79 cells (Tezuka et al., 1980) and for abnormal sperm morphology [illegible] mice (Osterloh et al., 1983).

Teratogenicity and Other Reproductive Effects. Acute, subchronic and chronic oral and inhalation exposures to DBCP have resulted in testicular atrophy and a reduction or loss of spermatogenesis in male laboratory animals. Discussion of these effects has been presented in the Effects of Acute Exposure, Effects of Subchronic and Chronic Exposure, and Effects on Reproductive Organs Sections and will not be repeated here.

The teratogenic effect of oral DBCP exposure in rats was studied by Ruddick and Newsome (1979). Effects of DBCP inhalation exposure on the fertility of male and female rats, on the rat fetus (Rao et al., 1983) and on the fertility of male rabbits (Rao et al., 1982) have also been reported.

Groups of 15 female Wistar rats that had been mated overnight (day one of gestation was indicated by the presence of sperm in the vagina on the next day) received DBCP (97.3% pure) in corn oil by gavage at doses of 0, 12.5, 25 or 50 mg/kg bw/day on days 6-15 gestation (Ruddick and Newsome, 1979) (see Table V-2). The dams were killed on day 22. No teratogenic effects were observed in fetuses at any dose level. Fetuses of dams at the 50 mg/kg bw/day dose level had significantly reduced mean body weight when compared with controls ($p<0.05$). Mean body weight was also significantly reduced compared with controls in dams that received 25-50 mg DBCP/kg bw/day

($p<0.05$ and $p<0.001$, respectively). One high-dose dam died on day 17 of gestation. The remaining 14 high-dose dams produced 10 litters compared with 13-14 litters produced by other treated and control groups. Upon necropsy, 3/4 high-dose dams that had failed to produce litters had edematous uteri containing a pink fluid. This finding suggested to the investigators that these rats had been pregnant, but the exposure to DBCP was fatal to the embryos.

In an extensive study on effects of DBCP inhalation exposure on fertility and other reproductive parameters of rats, Rao et al. (1983) failed to find any teratogenic or reproductive effects (see Tables V-7 and V-13). Groups of 20 female Sprague-Dawley rats (225-300 g, 6 months of age) were exposed to DBCP (97.3%) in air at concentrations of 0, 0.1, 1.0 or 10 ppm (0, 0.97, 9.7 or 97 mg/m³) 6 hours/day, 5 days/week for 14 weeks. At 1 or 2 weeks and 18-20 weeks after exposure was terminated, the female rats were mated with unexposed male rats and allowed to deliver at term. For the first or the second matings, no statistically significant differences were found between treated groups and controls with respect to the percentage of pregnancies, number of dead pups, number of live pups, 1-day survival or sex ratio. There were no statistically significant differences in pups born to treated dams compared with control pups with respect to the incidences of short tail, hematoma of the umbilical region, emaciation, runtism or hypothermia. The pups were not further examined. In the same study (Rao et al., 1983), male Sprague-Dawley rats (350-450 g, 6 months of age) were similarly treated by inhalation exposure to DBCP. At weeks 2, 4, 7, 10, 12 and 14 of exposure and at weeks 2, 5, 10, 15 and 27 after exposure, 20 males of each exposure level were mated with unexposed females. The mated females were killed 12 days after a 7-day cohabitation period. The females that were mated at 27 weeks postexposure were killed on day 21 of gestation (detection of vaginal plug or sperm in vaginal smear = day 0). Although male rats treated at 9.7 and 97 mg/m^3 levels had reduced sperm counts and loss of spermatogenic elements (see Subchronic Studies Section under Effects of Subchronic and Chronic Exposure), no significant differences were found between treated males and controls with respect to fertility index, defined as (number of fertile males ÷ number of males housed with females) x 100, for any mating. No significant differences were found between controls and unexposed females mated with exposed males with respect to mean number of implants/litter and mean resorptions/litter for any exposure or any mating. There were, however, consistently increased average numbers of resorptions/litter in dams mated to males exposed to 97 mg/m^3 from week 4 of exposure to week 5 after exposure. This was reflected in statistically significant differences in mean percent postimplantation loss, defined as (number of resorptions ÷ number of implantations) x 100, in these dams when compared with controls ($p<0.05$). Thus, DBCP was positive for dominant lethality in male rats (see Mutagenicity Section). The dominant lethal effect was not observed in later matings. For the unexposed dams that were mated to exposed males 27 weeks postexposure and killed at day 21 of gestation, no fetal abnormalities were detected.

Rao et al. (1982) also studied the reproductive effects of inhalation exposure to DBCP in male New Zealand rabbits. Groups of 10 male rabbits (6 months of age) were exposed to DBCP (97.3%) vapor at concentrations of 0, 0.1, 1.0 or 10 ppm (0, 0.97, 9.7 or 97 mg/m^3) for 6 hours/day, 5 days/week for 14 weeks. The exposures in the 97 mg/m^3 level rabbits were terminated after 8 weeks because of high mortality. The testicular and spermatogenic effects were discussed previously in Subchronic Studies Section under Effects of Subchronic and Chronic Exposure and Table V-7. The exposed rabbits were mated to unexposed females at week 14 and again at 27 weeks after exposure. The day of mating was considered to be day 0 of gestation. The dams were killed at day 28 or 29 of gestation. The results are summarized in Table V-14. For the 14-week mating, there was a significant decrease in total implantations/litter in the dams mated to 9.7 mg/m³ level males compared with controls. Male rabbits exposed at the 97 mg/m³ level were infertile. For the 27-week postexposure mating, fertility had been restored in 2/5 high-dose rabbits that were infertile in the first mating. These observations are in good agreement with observed loss of spermatogenesis in the high-dose male rabbits after 8 weeks of DBCP exposure and the partial recovery noted 33 weeks after exposure ceased (see Subchronic Studies Section under Effects of Subchronic and Chronic Exposure and Table V-7). Although there were 20% abnormal sperm after the recovery period in the high-dose rabbits (see Subchronic Studies Section under Effects of Subchronic and Chronic Exposure), no abnormal fetuses were found as a result of the 27-week postexposure mating.

Summary of Health Effects in Animals

Values for acute oral LD_{50} of DBCP varied from 170-350 mg/kg bw in rats, 260-410 mg/kg bw in mice, 210-316 mg/kg bw in guinea pigs and 180-440 mg/kg bw in rabbits (Torkelson et al., 1961; Rakhmatullayev, 1971). The dermal LD_{50} in rabbits was higher when DBCP was applied undiluted (1400 mg/kg bw) than when applied in propylene glycol (500 mg/kg bw) (Torkelson et

TABLE V-14

Fertility of Unexposed Female Rabbits Mated with DBCP-Exposed Male Rabbits[a]

	mg/m³ DBCP			
	0	0.97	9.7	97
	14-Week Mating			
No. of Males	9	10	9	5
% of males which copulated (no.)	100 (9)	90 (9)	78 (7)	100 (5)
% fertile males (no. fertile males/no. bred to females)	67 (6/9)	100 (9/9)	88 (6/7)	0 (0/5)[b]
Corpora lutea/dam[c]	10 ± 1	10 ± 3	8 ± 4	NA
Total implantation/litter[c]	9 ± 1	9 ± 1	6 ± 2[b]	NA
Resorptions/liter[c]	0.7 ± 0.8	0.9 ± 0.8	0.3 ± 0.5	NA
% Pre-implantation loss[c]	9 ± 15	14 ± 13	21 ± 20	NA
% Postimplantation loss[c]	8 ± 9	10 ± 9	4 ± 6	NA

TABLE V-14 (cont.)

	mg/m³ DBCP			
	0	0.97	9.7	97
	27-Week Postexposure Mating			
No. of males	6	6	5	5
% of males which copulated (no.)	83 (5)	100 (6)	80 (4)	100 (5)
% Fertile males (no. males impregnating one female/number bred)	100 (5/5)	100 (6/6)	100 (4/4)	40 (2/5)
% Fertile males (no. males impregnating two females/number bred)	80 (4/5)	100 (6/6)	100 (4/4)	20 (1/5)
Corpora lutea/dam[c]	10 ± 1	10 ± 2	10 ± 1	12 ± 3
Total implantations/litter[c]	8 ± 2	8 ± 2	8 ± 2	10 ± 6
Resorptions/litter[c]	0.7 ± 0.7	0.8 ± 0.5	0.2 ± 0.3	1.0 ± 1.4
% Pre-implantation loss[c]	24 ± 20	22 ± 17	19 ± 13	22 ± 26
% Postimplantation loss[c]	16 ± 24	15 ± 12	3 ± 3	7 ± 10

[a] Source: Adapted from Rao et al., 1982

[b] Significantly different from control, p<0.05

[c] Mean ± SD

NA = Not applicable

al., 1961). LC_{50} values for inhalation exposure in rats ranged from 3557 mg/m³ for 1 hour of exposure to 995 mg/m³ for 8 hours (Torkelson et al., 1961). DBCP was slightly less toxic to prepubertal mice than to adult mice when administered intraperitoneally (Lee and Suzuki, 1979). Acute high doses of DBCP (100-628 mg/kg bw) administered by gavage to rats resulted in biochemical changes, such as transiently decreased levels of cytochrome P-450 in liver, kidney and testes coinciding with decreased levels of heme (Moody et al., 1982a; Tofilon et al., 1980); increased hepatic protein and RNA content and changes in lipid components of microsomes (Moody et al., 1982b) and increased activities of the microsomal enzymes, aryl hydrocarbon hydroxylase and epoxide hydrolase (Suzuki and Lee, 1981). An acute lethal oral dose (400 mg/kg bw) to rats resulted in necrosis of hepatocytes and degeneration of renal tubules (Kato et al., 1980); a lower dose (100 mg/kg bw) resulted in reduced spermatogenesis (Reznik and Sprinchan, 1975). Oral doses of 40 or 50 mg/kg bw/day administered for 4 or 5 days resulted in decreased body weight, impaired renal function, necrotic lesions of liver and kidney and degeneration of testis and epididymis in rats (Kluwe, 1981a; Saito-Suzuki et al., 1982). Acute inhalation exposures of 483-6475 mg/m³ for up to 7 hours resulted in such effects as scarring of kidney tissue, pulmonary irritation, liver damage, CNS depression and death in rats (Torkelson et al., 1961; Kodama and Dunlap, 1956). Continuous inhalation of 29-97 mg/m³ DBCP for 14 days resulted in effects ranging from atrophy of seminiferous tubules, necrotic germ cells of testis, necrosis of proximal tubules in kidneys and necrotic lesions in lung epithelial tissue in rats (Saegusa et al., 1982). The dose-response for acute subcutaneous injection (DBCP was slightly more toxic by this route than by the oral route) of DBCP in rats was described by Kluwe et al. (1981). The no-effect level was 20 mg/kg bw. At ≥40 mg/kg bw, such effects as reduced body weight, impaired kidney function, degeneration of proximal renal tubule epithelium, hepatocellular necrosis and decreased spermatogenesis were observed.

The maximum tolerated subchronic gavage doses of DBCP were 24 mg/kg bw/day for male and female Osborne-Mendel rats and 160 and 120 mg/kg bw/day for male and female $B6C3F_1$ mice, respectively (NCI, 1977). Dietary administration of DBCP to rats for 90 days resulted in increased kidney weight at ≥2 mg/kg bw/day, reduced body weight gain at 15 mg/kg bw/day, increased liver weight and ruffled fur at ≥45 mg/kg bw/day, and muscular weakness and increased mortality at 135 mg/kg bw/day. The no-effect level was 0.5 mg/kg bw/day (Torkelson et al., 1961). Several reports from the Russian literature described effects in rats of subchronic exposure at low doses (0.05-5 mg/kg bw). Effects on hematologic parameters and renal and testicular function were observed (Rakhmatullayev, 1970). At higher doses, necrosis of liver, kidney and testis were observed (Rakhmatullayev, 1969; Faydysh et al., 1970; Reznik and Sprinchan, 1975). Effects of chronic gavage exposure to DBCP in a carcinogenicity bioassay included dose-related increased mortality and a high incidence of toxic tubular nephropathy in mice and rats (NCI, 1977). These effects were observed for TWA doses of 10.7-146 mg/kg bw/day.

Commonly observed effects of subchronic inhalation exposure were testicular atrophy and reduced spermatogenesis in male animals (Torkelson et al., 1961; Rao et al., 1982, 1983). Lesions of the upper respiratory system and nasal cavity occurred in rats and in mice (NTP, 1982a; Resnik et al., 1980a,b,c,d). Reduced body weight gain and increased mortality occurred at high exposure levels (48-483 mg/m³) (Torkelson et al., 1961; NTP, 1982a; Rao et al, 1983). Histopathologic changes in kidney tubules, liver, testis and adrenal cortex of rats have been observed (Torkelson et al., 1961; Rao et al., 1983). At inhalation exposures of ≥1 ppm (9.7 mg/m³) 6 or 7 hours/day, 5 days/week for up to 14 weeks in rats and mice, dose-related effects ranged from decreased body weight, increased liver weight and focal histopathologic changes in testis, renal tubule, lung and nasal cavity to increased mortality and extensive necrosis (NTP, 1982a; Torkelson et al., 1961). A no-effect level for mortality, clinical chemistry, hematology, body weight, organ weights and testicular effects was reported for rats and rabbits as 0.1 ppm (0.97 mg/m³) 6 hours/day, 5 days/week for 14 weeks (Rao et al., 1982, 1983). Effects of chronic inhalation exposure to DBCP in a carcinogenicity bioassay included dose-related decreased mean body weight, increased mortality, increased incidences of toxic tubular nephropathy and histopathologic lesions of the nasal cavity and stomach in rats and mice at concentrations of 5.8 and 29 mg/m³ (NTP, 1982a).

DBCP has been studied for carcinogenicity in mice and rats by oral and inhalation routes and in mice by dermal application. An NCI (1977) bioassay reported highly significant dose-related increased incidences in Osborne-Mendel rats of squamous-cell carcinoma of the forestomach in males and females and mammary adenocarcinoma in females receiving chronic gavage corrected TWA doses of 10.7 and 20.7 mg/kg bw/day. Significant dose-related increased incidences of squamous-cell carcinoma of forestomach of male and female $B6C3F_1$ mice were found for chronic gavage corrected TWA doses of 78.6-149.3 mg/kg bw/day. In a chronic dietary carcinogenicity bioassay in rats conducted by Hazelton Laboratories (1977) and also reported by U.S. EPA (1979a; 44 FR 65135), high-dose (2.0 mg/kg bw/day) male and female rats had significantly increased incidences of carcinoma of the renal tubules and squamous-cell carcinoma of the stomach. High incidences of stomach squamous-cell carcinoma were observed in high-dose male and female mice as well. An NTP (1982a) bioassay reported dose-related increased incidences of nasal cavity tumors in male and female F344 rats and $B6C3F_1$ mice receiving chronic inhalation exposures to DBCP at concentrations of 5.8 and 29 mg/m^3, 6 hours/day, 5 days/week. The mice also had treatment-related increased incidences of pulmonary tumors. DBCP was positive as a tumor initiator on the skin of HA:ICR Swiss mice but negative as a whole carcinogen for skin (Van Duuren et al., 1979). In the whole carcinogen assay, however, the incidence of distant tumors of lung and stomach were significantly increased over controls.

Technical DBCP was mutagenic in S. typhimurium strains TA1535, TA1530, TA100 and TA98 and in E. coli with and without metabolic activation (Prival et al., 1977; Rosenkranz, 1975, Stolzenberg and Hine, 1979; Moriya et al., 1983). Some of the mutagenic potential of DBCP has been attributed to epichlorohydrin (Biles et al., 1978), a contaminant as well as a metabolite of DBCP. Negative results were obtained with S. typhimurium strains TA1537 and TA1538 (Rosenkranz, 1975; Moriya et al., 1983). DBCP was positive in the recessive lethal assay, in a genetic crossing over assay and for chromosome breakage in D. melanogaster (Inoue et al., 1982; Zimmering, 1983; Kale and Baum, 1982b). Dominant lethal assays were positive in rats (Teramoto et al., 1980; Saito-Suzuki et al., 1982; Rao et al., 1979, 1983) but negative in mice (Teramoto et al., 1980). Positive results were obtained for sister

VI. EFFECTS IN HUMANS

Case Studies

No case studies of acute exposure to DBCP were found in the available literature. The only reported effects of acute exposure to DBCP in humans were organoleptic. Rakhmatullayev (1969) reported that the taste and smell thresholds in water were 0.01 mg/ℓ. Two human volunteers were placed in a chamber containing 1.7 ppm (16.4 mg/m³) DBCP long enough (time not specified) for then to detect a "definite, not unpleasant odor" (Torkelson et al., 1961). According to Dow Chemical Co. (42 FR 48026) humans can detect 0.01-0.03 ppm (0.097-0.29 mg/m³) of DBCP in air.

Epidemiological Studies

Although DBCP exposure has resulted in stomach, mammary and respiratory system tumors (see Carcinogenicity Section in Chapter V) and toxicity to the kidneys and several other organs (see General Toxicity and Target Organ Toxicity Sections in Chapter V) in rats and mice, no association between DBCP exposure and cancer or renal effects in humans has been documented.

One of the most consistent findings in laboratory animals exposed to DBCP is decreased sperm counts and interference with spermatogenesis (see General Toxicity and Target Organ Toxicity Sections in Chapter V). The discovery in 1977 that several workers of the Occidental Chemical Company in California, a plant that produced DBCP as well as other chemicals, were azoospermic (sperm count of 0 million/mℓ) (Whorton et al., 1977), prompted several epidemiological studies on the antispermatogenic effect of DBCP. These studies fall into two major categories: studies of chemical production plant workers and studies of agricultural workers potentially exposed to DBCP. The major findings of these studies are presented in Tables VI-1 and VI-2, respectively. Supplemental information is supplied in the text. For the studies in general, efforts were made to identify all employees who were potentially exposed to DBCP, and participation was on a voluntary basis. In addition to information obtained from employment records, personal and medical histories with an emphasis on histories of reproduction were obtained from the study subjects. In each case, the participants were given physical examinations and instructed as to semen collections, which were usually obtained after 2 or 3 days of sexual abstinence. Blood samples were also taken for hormone level determinations and testicular biopsies were performed in a few studies. For all discussions, oligospermia (low sperm count) is defined as <20 million sperm/mℓ of semen (MacLeod and Gold, 1959).

Studies of Chemical Company Employees. Whorton et al. (1977) conducted a study on infertility in workers of the Agricultural Chemical Division of the Occidental Chemical Company in California after several workers had complained of inability to have children. The airborne concentrations of DBCP were believed to be <1 ppm (9.7 mg/m^3). Measurement of 0.4 ppm (3.7 mg/m^3) was made early in 1977 by personal air-sampling device. Another report of this study has been presented in a separate publication (Marshall et al., 1978). Members of the original cohort of 39 employees included supervisors, production workers, clerks, maintenance and laboratory personnel. There were 36 men, 11 of whom had had vasectomies. All three women in the original cohort had normal menstrual cycles, had given birth to children and were eliminated from the study. None of the 36 men had sexual dysfunction or abnormalities of the genitalia or accessory organs. Varicoceles was

TABLE VI-1

Decreased Sperm Count, Elevated Hormone Levels and Related Effects in Production Plant Workers Occupationally Exposed to DBCP

Study Population	Control Population	Duration of Exposure	Level of Exposure	Effects	Reference
22 male workers of Occidental Chemical Corp. plant in California: Group A - 11 men with mean age 32.7±1.6 years; Group B - 11 men with mean age 26.7±1.2 years	no controls	8.0±1.2 years 0.08±0.02 years	not quantified but airborne concentration believed to be <1 ppm (<9.7 mg/m³)	Significant differences were found between the two groups for age ($p<0.01$) and duration of exposure ($p<0.001$). For the men exposed for 8 years, mean sperm count was 0.2±0.1 million/mℓ; FSH level was 11.3±1.8 MIU/mℓ; LH was 28.4±3.3 MIU/mℓ and testosterone was 459±35 ng/dℓ. For the men exposed for 0.08 years mean sperm count was 93±18 million/mℓ, FSH was 2.6±0.4 MIU/mℓ; LH was 14.0±2.8 MIU/mℓ and testosterone was 463±31 ng/dℓ. All results are expressed as mean ± SD. The two groups were significantly different with respect to FSH ($p<0.001$) and LH ($p<0.01$). No p value was given for sperm count differences; however, the difference is striking and greater than could be accounted for by the age difference. Nine of the 11 men of Group A were azospermic. The 2 oligospermic men had reduced sperm motility and increased incidence of abnormal sperm.	Whorton et al., 1977
107 nonvasectomized employees of Occidental Chemical Plant in California including the 22 men in the above study and applicators and demonstrators working at sites remote from the plant Ages NR	35 nonvasectomized employees of the plant with no DBCP exposure Ages NR	1-42 months	not quantified but airborne concentration believed to be <1 ppm (<9.7 mg/m³)	Median sperm count: exposed group - 4.6×10^7 control group - 7.9×10^7 Azoospermia in 14/107 (13%), oligospermia in 18/107 (16.8%) and low normal sperm count (20-39 million/mℓ) in 17/107 (15.8%) of exposed men. Frequency distribution was not reported for the controls. In a subgroup of 91 DBCP exposed men (16/107 could not be classified as to duration) the incidence of oligospermia was 4/48 (8%) exposed 1-6 months; 4/14 (29%) exposed for 7-24 months; 8/12 (67%) exposed for 24-42 months and 14/17 (82%) exposed for >42 months. For the controls oligospermia occurred in 1/35 (3%).	Whorton et al., 1979

TABLE VI-1 (cont.)

Study Population	Control Population	Duration of Exposure	Level of Exposure	Effects	Reference
				The controls of multiple regressions correlating log sperm count, duration of exposure, age and levels of testosterone, FSH and LH indicated that log sperm was inversely related to FSH and LH and to duration of exposure (grouped by quarter years), and FSH was directly related to LH. No other correlations were significant.	
91 male DBCP-exposed workers at a Shell Oil Company production plant in Denver, Colorado	Internal Control 29 nonexposed male workers of the plant: 9 had been vasectomized, 20 had not	≤19.5 years	airborne concentrations NR	For the exposed group (not including vasectomized men): mean sperm count = 65.23±50.95 million/mℓ; 7% were azoospermic; 21.9% were oligospermic; FSH levels = 328.5±741.5 (no units given). For the internal group: mean sperm count = 74.45±54.55 million/mℓ; 0% were azosopermic, 10% were oligospermic and FSH level was 160.2±74.55 (no units given).	Lipshultz et al., 1980
27 men had been vasectomized, but participated in hormone level studies; 64 men were nonvasectomized and participated in hormone level and semen analyses	Mean age = 29.8 years			No significant difference between groups for sperm count distribution when analyzed nonparametrically using Kolmogorov-Smirnov test or Mann-Whitney U test. No difference was found by t-test for mean sperm count when normalized by taking mean log (sperm count +20).	
Mean age: 38.7 years	External Control 87 male medical students			The groups were significantly different with respect to FSH level when tested nonparametrically by both tests and by t-test when data were normalized by taking mean log (FSH) ($p<0.00041$). At weighted exposure hours (see text) of >1000 hours, mean log (sperm count +20) was significantly different from control ($p=0.0401$). When compared nonparametrically with medical students, sperm count frequency distribution of exposed men was significantly different ($p<0.001$).	

TABLE VI-1 (cont.)

Study Population	Control Population	Duration of Exposure	Level of Exposure	Effects	Reference
71 DBCP-exposed male workers in a Shell Oil Company production plant in Mobile, Alabama (none had been vasectomized) Mean age= 28.7 years	Internal Control 37 nonexposed male workers of the plant: 3 had been vasectomized but participated in hormone level studies; 34 were studied for semen and hormone analyses Mean age= 28.6 years External Control 87 male medical students	<1 year	airborne concentrations NR	For the exposed group: mean sperm count = 56.6 ± 44.1 million/mℓ; 2% were azoospermic, 16.9% were oligospermic; FSH level was 187.9 ± 114.5 (no units given). For the internal control group: mean sperm count = 96.6 ± 66.5 million/mℓ; 0% were azoospermic; 8.8% were oligospermic and FSH was 141.88 ± 73.78 (no units given). The two groups differed significantly with respect to sperm count distribution by nonparametric Kormogorov-Smirnov ($p<0.002$) and Mann-Whitney U tests ($p<0.000076$) and with respect to FSH levels by the latter test ($p<0.0529$). Mean log (sperm count +20) and mean log (FSH) were signficantly different ($p=0.00151$ and $p=0.0177$, respectively) between the groups by t-test. Mean log (sperm count +20) was significantly different from control at weighted hours of exposure (see text) of <100 hours ($p=0.0073$); <300 hour ($p=0.00628$); <500 hours ($p=0.0025$); >100 hours ($p=0.00167$); >200 hours ($p=0.0021$) and >300 hours ($p=0.00048$), but was not significantly different in the group exposed <40 hour ($p=0.0585$). Sperm counts distribution of exposed was significantly different compared with 87 medical students ($p<0.02$).	Lipshultz et al., 1980
14/15 oligospermic men from the Mobile, Alabama cohort in the preceding study Mean age: 27.1 years Range: 20-33 years Group I = 7 men with extratesticular abnormalities (e.g., varicocele, epididymal obstruction)	10 normal men of "similar ages" from the investigators laboratory	<50-700 weighted hours (see text)	airborne concentrations NR	Sperm counts were determined 6-12 months after last DBCP exposure. Group I: range - 0-13 million/mℓ; mean ± SE = 4.8 ± 2.0 million/mℓ. Group II: range - 1.3-27.6 million/mℓ; mean ± SE = 13.8 ± 3.2 million/mℓ. Significant differences from control were found for reduced carnitine levels (a marker for epididymal function) and reduced testosterone level. The glycerylphosphorylcholine level (marker of epididymal function) was significantly reduced for Group I and the FSH and LH levels were significantly elevated for Group II when compared with control ($p<0.05$).	Lantz et al., 1981

TABLE VI-1 (cont.)

Study Population	Control Population	Duration of Exposure	Level of Exposure	Effects	Reference
Group II = 7 men without abnormalities				To study recovery, time since last DBCP exposure was divided into 1-6 months, 6-12 months and 12-18 months. The mean sperm count at 12-18 months for Group I and II is significantly different from mean sperm count at 1-6 months for Group I and II ($p<0.02$).	
				Testicular biopsies at 1-6 months after exposure (for both groups) revealed late spermatids, sparcity of germ cells, focal hyalinization of tubule walls, markedly reduced spermatogenesis; some tubules contained only Sertoli cells.	
232 male employees of Dow Chemical plant in Midland, Michigan	97 male employees at the plant with no known DBCP exposure		not quantified airborne concentrations monitored in 1974 and 1975 indicated <1 ppm (<9.7 mg/m^3) 8-hour TWA	The sperm count frequency distribution was not statistically analyzed. In general low sperm counts and reduced testicular volume were observed more frequently in the exposed than in control workers. No mean sperm counts were reported; oligospermia was observed in 16/113 (14%) production workers, 3/41 (7%) of strike workers, 20/75 (27%) of nonproduction workers and 10/97 (10%) of controls.	Egnatz et al., 1980
114 worked in production		≤18 years			
41 had worked in production during 6 month strike		≤6 months			
77 were nonproduction workers (maintenance quality control)		≤18 years		Mean FSH ($p<0.05$) and LH ($p<0.01$) were significantly elevated in the production group and LH was elevated in nonproduction group compared with controls ($p<0.05$).	
Ages: NR				The workers were subdivided as to time since last DBCP exposure, either <5 or >5 years and exposure category (direct, indirect, intermittent, distant) and five response variables (sperm count, testicular volume, FSH, LH and testosterone levels) were analyzed statistically. Of 60 comparisons, 51 were not significant. Only in production workers directly exposed with a <5 year period since last DBCP exposure was more than 1 variable significant; sperm count ($p<0.01$), FSH ($p<0.005$) and testes volume ($p<0.005$).	

TABLE VI-1 (cont.)

Study Population	Control Population	Duration of Exposure	Level of Exposure	Effects	Reference
23 male employees of a DBCP production line of a chemical plant in Israel Mean age ± SD = 28.9±9.5 years (range 19-50 years)	there were no controls for sperm count controls for hormone levels were normal males in the laboratory	100-6726 hours (obtained from plant records)	airborne concentrations NR	The 23 men were divided as to sperm counts.	Potashnik et al., 1979; Potashnik, 1983
				Azoospermia: 12/23 men (20-50 years of age), last exposure was 1-5 years before study for 5 of the 12, 8 had previously sired children, the other four had normal karyotypes. Increased plasma FSH levels (13.4-30.0 MIU/mℓ) above upper limit of normal (10 MIU/mℓ), 1/12 had elevated LH and decreased testosterone levels. Histology: atrophy of seminiferous tubules, only Sertoli cells in many tubules; no spermatogenic elements, hyalinization of tubules.	
				Four years later: 3 of original 12 plus 1 worker not included in first study, had recovery of sperm counts (3 men had <8 million/mℓ, 1 had 40 million/mℓ). The duration of exposure had been 114-390 hours. Recovery time (time between last DBCP exposure and first improved count), 20-45 months. Two had successfully sired children. Of original 12, 9 remained azoospermic and FSH and LH levels were 1.5-3 times higher than previous 4 years.	
		34-97 hours		Oligospermia: 6/23 men had counts 0.5-10 million/mℓ; ages ranged from 19-37 years. Time since last DBCP exposure was 2-24 months; 3 were married; 2 fathered children. FSH, LH and testosterone levels were within normal limits. In 2 biopsies, histological findings were similar to azoospermics; however, active spermatogenesis was detected.	
				Four years later: 4 of original 6 men plus 1 not included in original study had recovered, sperm counts were appreciably elevated compared with 4 years previously; 3 still had counts <20 million/mℓ. Recovery time was 14-36 months. All 5 men with improved counts had successfully sired children. Exposures had been <79 hours. The 2 men whose counts did not improve had been exposed for 220 and 310 hours, respectively.	
		10-60 hours		>20 million/mℓ: 5/23 men (22-26 years of age) had counts of 20.5-65.0 million/mℓ at 2 months since last exposure. Hormone levels were within normal limits.	

TABLE VI-1 (cont.)

Study Population	Control Population	Duration of Exposure	Level of Exposure	Effects	Reference
18 male employees exposed to DBCP Mean age: 33.8 years	15 nonexposed men Mean age: 33.0 years	Mean: 15.2 months Range: 6-18 months	not quantified airborne concentration estimated at <1 ppm (<9.7 mg/m^3)	Nondisjunction of y-chromosome was indicated by the appearance of 2 fluorescent y-chromosomes (YFF) in a normal sized spermatozoon after treatment with quinacrine dihydrochloride. Normal sperm would contain 1 YF body. The mean % YF (normal) was similar for both groups (41.5 and 41.8) but mean % YFF (nondisjunction) was 3.8 (range: 2.0-5.3%) for exposed and 1.2 (range: 0.8-1.8%) for nonexposed groups. Background frequency of YFF, determined in 262 semen samples from other men was 1.3%. Since the investigators considered <2% YFF frequency as normal, all 15 controls had normal frequency; only 2/18 DBCP-exposed men had normal frequency (x^2 = 22.5; $p<0.001$).	Kapp et al., 1979

NR = Not reported

TABLE VI-2

Decreased Sperm Counts, Elevated Hormone Levels and Related Effects in Agricultural Workers Occupationally Exposed to DBCP

Study Population	Control Population	Duration of Exposure	Level of Exposure	Effects	Reference
South Carolina Cohort 53 agricultural workers from South Carolina, Florida, Arkansas, Tennessee and Mississippi, employed as formulators, applicators, farmers and farm hands, salesmen and researchers Ages ranged from 20-58 years	9000 men with no known exposure to toxic chemicals from an independent study of fertility[a]	1-1458 days of lifetime use (total number of days of individual use)	use indices ranged from 0-13,500 lb/day "Use Index" = total number of pounds used in lifetime ÷ total number of days of use	Sperm counts categories were <10, 10.1-20, 20.1-40, 40.1-60, 60.1-100 and >100 million/mℓ. Statistically significant difference in frequency distribution in sperm count categories between 9000 nonexposed men and 8 formulators (X^2=24.0; $p<0.001$); 43 other users (applicators, farmers, farm hands, researchers and salesmen) (X^2=19.0, $p<0.005$) and formulators plus users (X^2=32.1, $p<0.001$). Azospermics (2/45) were excluded from comparison. Incidence of oligospermia 6/8 (75%) formulators, 13/43 (32.5%) users and 1350/9000 (15%) of controls. Azoospermics were excluded from comparison with controls. Formulators, applicators and farmers had lower median sperm counts and higher hormone levels than research workers. Salesmen had intermediate values. Negative correlation between sperm count and FSH and LH was significant ($p<0.0001$). Use indices of ≤400, 400.1-800 and >800 were compared with respect to frequency distribution among sperm count categories. Spearman Rank Correlation Coefficient = -0.372 ($p<0.01$). Thus, the greater the index the lower the sperm count.	U.S. EPA, 1978; Sandifer et al., 1979
California Cohort 72 male agricultural workers (applicators, farm workers, researchers) Ages ranged from 20 to >50 years	23 male agricultural workers who had no DBCP exposure Ages ranged from 20 to >50 years	ranged from possible incidental to ~2000 days of lifetime use	ranged from unknown to ~3000 lb/day "Use Index" = total number of pounds used in lifetime ÷ total number of days of use	For 15 men with >500 days of use, 35.7% were oligospermic, 6.7% were azoospermic. For 18 men with 101-500 days of use, 41.2% were oligospermic and 5.6% were azoospermic. At <100 days of use, 0% azoospermia was found; the percent oligospermia declined with declining duration. Median sperm count increased as numbers of days of use decreased. For 60 exposed men (excluding 12 with possible incidental exposure) 21/60 were oligospermic compared with 1/23 (4.3%) ($p<0.005$)[b]. There was a significant negative correlation between sperm count and FSH and LH levels ($p<0.01$; $p<0.05$, respectively).	U.S. EPA, 1978

TABLE VI-2 (cont.)

Study Population	Control Population	Duration of Exposure	Level of Exposure	Effects	Reference
Texas Cohort 14 agricultural workers (farmers, irrigators and farm hands) Mean age: 44.7 years Range: 26-70 years	9000 men with no known exposure to toxic chemicals from an independent study of fertility[a]	3-540 days of lifetime use	ranged from 24-1100 lb/day "Use Index" = total number of pounds used in lifetime ÷ total number of days of use	Mean sperm count - 38.9 million/mℓ Median sperm count - 31.8 million/mℓ % azoospermic - 0 Frequency distribution of counts: <20 million/mℓ - 6/14 (42.9%) 20-40 million/mℓ - 2/14 (14.2%) >40 million/mℓ - 6/14 (42.9%) For incidence oligospermia (42.9%) and for total counts <40 million/mℓ (8/14 or 57.1%) in exposed men compared with the incidence in 9000 control men (18.1 and 29.1%, respectively) p<0.025 for each case[b]. The correlation between reduced sperm count and increased FSH and LH was not signficant.	U.S. EPA, 1978
74 male professional applicators of DBCP from 20 firms in 5 counties of California Ages NR	22 male professional applicators never exposed to DBCP Ages NR	1 day to >2 months in the study year; exposures had also occurred in 1 to >8 years previous	not quantifiable	Analysis of variance: Negative exposure-related trend in mean log sperm count for men exposed during the current year (p=0.018). The mean log sperm counts were compared for men exposed for 0 days, 1 day to 2 weeks, for >2 weeks to 2 months and for >2 months. Only mean log sperm count for >2 months exposure was significantly different from others (no p value given). Azoospermia in 4/24 (17%) men in this group. Negative trends for mean log sperm count for workers exposed in 1-8+ past years was not significant and confounded by more recent exposure. There was a positive exposure-related trend for mean FSH (p<0.05) for the men exposed in year of study.	Glass et al., 1979
13 pineapple workers on the island of Molokai, Hawaii	14 men of general population of Molokai	mean - 2.8 years with median frequency of 6 days/year	not quantifiable	For the exposed group, mean sperm count was 33.5 million/mℓ; 23% had oligospermia. For the control group, mean sperm count was 84.9 million/mℓ and 14% were oligospermic.	Takahashi et al., 1981

TABLE VI-2 (cont.)

Study Population	Control Population	Duration of Exposure	Level of Exposure	Effects	Reference
Mean age: 37.6 years Range: 23-51 years 1 worker smoked 1 marijuana cigarette/week	Mean age: 32.4 years Range: 21-49 years 5 controls smoked marijuana with a frequency of <2 cigarettes/week Also 72 fertile men of Honolulu from an independent study on fertility[c]			Analysis of frequency distribution of sperm counts comparing: exposed workers with control (Molokai - $X^2=8.13$; $p<0.02$; exposed workers with controls (Molokai) plus 72 fertile men - $X^2=364$, $p<0.001$). The frequency distribution of the workers vs. the 72 fertile men of Honolulu were highly statistically significant. The z statistic was 15.43. Similar comparison of workers vs. 72 infertile men and Molokai controls vs. 72 fertile men were not significant.	Takahashi et al., 1981
62 married male banana plantation workers from 14 kibbutzim in the Jordan Valley, Israel	the same 62 men before DBCP exposure	1-20 growing seasons	not quantifiable	For the 62 couples, the incidence of spontaneous abortion per total pregancies was 5/76 (6.6%) before the husbands had been exposed to DBCP compared with 24/121 (19.8%) after the initial exposure ($X^2=5.52$; $0.01<p<0.025$). The mean ages of the mothers were 25.6 years (before) and 28.3 years (after). In 16 of the 62 couples, the wives became pregnant both before and after DBCP exposure in the husband. The incidences of spontaneous abortion were 2/34 (5.9%) before and 11/40 (27.5%) after ($X^2=4.6$; $p<0.05$). The mean ages of the mothers were 23.8 years (before) and 30.2 years (after).	Kharrazi et al., 1980

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[a] MacLeod and Wang, 1979

[b] This comparison and statistical analysis was reported by Kemper and Bernstein (1978).

[c] Rogers et al., 1979

observed in three men who had previously sired children. Of the 36 men, 29 had fathered children. The 11 men who had vasectomies and 3 men with sperm counts between 10 and 30 million/mℓ were excluded from the study, leaving 11 men with ≤1 million sperm/mℓ and 11 men with ≥40 million sperm/mℓ. The two groups were then compared with respect to number of years they had been exposed. The results are presented in Table VI-1. All the workers in Group A had been exposed to DBCP for ≥3 years and all Group B men had been exposed for ≤3 months. Consistent with these findings is that the 3 men with intermediate counts had been exposed for 1-3 years. The results of this study prompted the investigators to conduct an epidemiological study of testicular function of all male employees of the plant (Whorton et al., 1979).

In this expanded study of 310 male subjects at risk (261 plant employees, 15 applicators or demonstrators and 34 salaried employees), 196 were examined including the original 36 from the 1977 study. It was assumed that only the 154 employees who worked in the Agricultural Chemical Division, the pellet plant and in field application were potentially exposed to DBCP. The remaining 42 men constituted the control population. Semen samples were obtained from 107 exposed and 35 control men after 48 hours continence. The results, presented in Table VI-1, indicated that reduced sperm count was directly related to length of DBCP exposure, although the results of statistical analyses were not presented. Other chemicals produced at the plant included epichlorohydrin, ethylene dibromide (also suspected of decreasing sperm counts), carbaryl, diazinon, dinoseb, endosulfan, malathion, maneb, methyl parathion, parathion, toxaphene and zineb.

Histological findings of testicular biopsies in 10 volunteers from the Whorton et al. (1979) study were presented in a separate report (Biava et al., 1978). In general, subjects with the least exposure (~2 years) had normal or only slightly reduced sperm production with normal appearing germ cells and Sertoli cells; subjects with ≥10 years exposure were azoospermic. Seminiferous tubules contained Sertoli cells that appeared normal but were devoid of germ cells. Subjects with intermediate exposure were azospermic to oligospermic and only a few tubules contained germ cells. No DBCP-exposure-related abnormalities other than absence of spermatogenic elements were noted in any of the biopsy samples.

The finding of reduced sperm counts in employees of the Occidental Chemical Company prompted epidemiologic surveys of DBCP-producing plants. Lipshultz et al. (1980) conducted studies in workers of two Shell Oil Company production plants -- one in Denver, Colorado, the other in Mobile, Alabama. For the Denver plant, which had produced DBCP from 1956 to 1976, 291 men were employed at the time of the study; 182 of these men had been exposed to DBCP, and 91 of the 182 participated in the study. Only 29 of 109 nonexposed men participated. Results are presented in Table VI-1. The workers were classified for exposure by a method whereby the number of exposure hours was multiplied by an empirical weighting factor, the magnitude of which reflected the intensity of exposure of the individuals' job classification, thus giving a value for weighted hours of exposure. To investigate whether a minimum exposure (threshold) was required to produce an effect on the mean log (sperm density + 20) where sperm density was expressed in 10^6/mℓ, the data were placed in overlapping groups according to weighted hours of exposure: e.g., <40, <100, <300, <500, and >100 to >1000 hours.

Only at >1000 weighted hours was mean log (sperm count + 20) significantly different from control when tested by the modified t test for differences between means with unequal variances (1 tail) (p=0.0481). One unusually high data point was eliminated from analysis with the modified t test. The lack of significant differences in sperm counts between the exposed and the control group may have been due to recovery of spermatogenesis in some of the exposed individuals, since no exposures occurred between 1976 and the date on which determinations were made, which was not specified, but was later than July, 1977. Partial recovery of spermatogenesis after exposure to DBCP ceases has been reported for rabbits (Rao et al., 1982). Because the nonexposed group was of a much smaller size than the exposed group, the sperm count frequency distribution of the exposed Denver workers was compared with the distribution in 87 medical students. The difference was highly significant (p<0.001) (Lipshultz et al., 1980).

For the plant in Mobile, Alabama, which had produced DBCP for only ~1 year (1976 to July, 1977), 169 men were employed when DBCP production was terminated (Lipshultz et al., 1980). Of these, 85 men had been exposed, 71 of which participated. None of the exposed men had had vasectomies. The nonexposed group consisted of 84 men, 37 of which participated. Results are given in Table VI-1. The differences in sperm count distribution between exposed and nonexposed, and differences in mean log (sperm count + 20) with all weighted hours of exposure were highly significant by all statistical tests employed, except when those individuals exposed <40 weighted hours were compared with the nonexposed group.

The differences between the results of the cohort in Denver, Colorado, and the cohort in Mobile, Alabama, may be due to differences in the time periods since cessation of exposure of DBCP. The men at Mobile had been exposed to DBCP more recently and, hence, their sperm counts were depressed. Some men at the Denver plant may have had time for spermatogenesis to recover. Of 15 oligospermic men that had been exposed to DBCP at the plant in Mobile, Alabama (Lipshultz et al., 1980), 14 had consented to undergo further study. The results, which were discussed in a separate report (Lantz et al., 1981), are presented in Table VI-1. Mean sperm counts for five men from each group appeared to recover as the time since the last DBCP exposure increased but the recoveries were not statistically significant. When the two groups were combined, the mean sperm counts were higher at 12-18 months after the last exposure than at 1-6 months after the last exposure ($p<0.02$).

In an epidemiological survey conducted at a Dow Chemical Company plant in Midland, Michigan (Egnatz et al., 1980), the medical examinations of the study and control populations were conducted at least 20 months since the last DBCP exposure (see Table VI-1). The plant had produced DBCP from 1957 to 1975, but airborne levels were monitored only during 1974 and 1975. Eight-hour TWA exposure levels for these 2 years were reported as <1 ppm (<9.7 mg/m^3). The investigators considered this information to be inadequate to quantify exposure for the entire period. In general, low sperm counts, reduced testicular volume, and high FSH and LH levels were observed more frequently in exposed groups than in nonexposed groups. Production workers with the most direct exposure to DBCP were the most affected. When workers were subdivided by exposure category (direct, indirect, intermittent, distant) and time period since last exposure (<5 years and >5 years), few statistical correlations with response variables (sperm count, FSH levels, etc.) were found among workers directly, indirectly, intermediately or distantly exposed. For production workers directly exposed with <5 years since last DBCP exposure, decreased sperm count, increased FSH level and decreased testicular volume were significantly related to exposure. These findings support the observations that the testicular effects of exposure to DBCP are greatest when exposure is more intense and more prolonged. The findings also support observations that testicular function partially recovers after DBCP exposure is terminated. Further support for these observations was reported by Potashnik et al. (1979) and Potashnik (1983).

After finding azoospermia in six Israeli factory workers involved in DBCP production (Potashnik et al., 1978), Potashnik et al. (1979) studied testicular function in a group of 23 male employees (including the original 6) of a chemical plant in Israel. The results are presented in Table VI-1. It was not reported whether the 23 men represented all of the individuals at risk. Statistical analyses of the results were not performed. Nevertheless, 18 of the 23 men (78%) with known histories of DBCP exposure had low sperm counts. Histological examination of testicular biopsy samples indicated a reduced number of spermatogenic elements in the oligospermics and absence of spermatogenesis in azoospermics.

In a follow-up study 4 years later, Potashnik (1983) demonstrated that azoospermia and oligospermia were reversible and the reversibility was influenced by the intensity and duration of exposure as well by the recovery period. The follow-up study was conducted in 17 of the original 23 subjects. In addition, three subjects, not included in the 1979 study, were followed for the 4-year period. Thirteen of the 20 subjects in the 1983 study had been azoospermic in the Potashnik (1979) study. The results are presented in Table VI-1. For the men who had been azoospermic, 4/13 (31%) had recovered sperm counts; for the men who had been oligospermic, 5/7 (71%) had improved counts. The azoospermic men had been exposed ~10 times longer than the oligospermic, but recovery times were shorter for the oligospermics than the azoospermics.

Kapp et al. (1979) reported genetic toxicity of DBCP in 18 male employees of a DBCP production plant (see Table VI-1). The mean frequency of nondisjunction of the y-chromosome was significantly increased over background in the exposed men compared with the mean frequency of nondisjunction in 15 controls ($p<0.001$).

Studies in Agricultural Workers. Concern over the finding of antispermatogenic effects in chemical production workers has prompted studies on the effects of occupational exposure in agricultural workers to DBCP (see Table VI-2).

An extensive epidemiologic survey of agricultural workers exposed to DBCP was initiated in 1977 by the Human Effects Monitoring Branch of the Office of Pesticide Programs (U.S. EPA, 1978). Four cohorts, the South Carolina Cohort, the California Cohort, the Texas Cohort and the New Jersey Cohort were studied for testicular effects.

The South Carolina Cohort consisted of 73 men ranging in age from 20-58 years from six southern states (U.S. EPA, 1978). A separate report of results in this cohort has been published (Sandifer et al., 1979). Analyses of the results excluded 20 men who had vasectomies. The results, presented in Table VI-2, indicated that persons with jobs requiring direct exposure and long durations of exposure to DBCP, had lower sperm counts than less intensely exposed workers when compared with 9000 men of an independent fertility study (MacLeod and Wang, 1979). The Use Index (total number of pounds of DBCP used in a lifetime + the total number of days of use) was significantly correlated with the frequency distribution of sperm counts ($p<0.01$).

The California Cohort consisted of 72 male agricultural workers potentially exposed to DBCP and 23 male agricultural workers with no exposure to DBCP. The results of sperm count analyses are presented in Table VI-2. Greater incidences of oligospermia occurred in the exposed workers than in the controls. The percentage of workers with low sperm counts declined as the duration of exposure declined.

In the Texas Cohort, the incidence of low sperm counts was significantly greater in 14 agricultural workers who had been exposed to DBCP than in 9000 men of the MacLeod and Wang (1979) study ($p<0.025$) (see Table VI-2).

The New Jersey Cohort consisted only of four agricultural workers with exposure to DBCP (U.S. EPA, 1978). Results of this study are not included in Table VI-2. Ages ranged from 20-44 years; the number of days of use varied from 3 to 15; the maximum use index was 333 lb/day; and sperm counts were between 38 and 105 million/mℓ. No comparison with controls was made.

Glass et al. (1979) studied the effects of DBCP exposure on fertility and spermatogenesis in pesticide applicators selected from 20 participating professional firms in five counties of California. Of a total of 112 men, 101 were interviewed. Semen samples were provided by 96 men, 74 of whom had been exposed to DBCP. There was no relationship between clinical infertility, defined as one year of unprotected coitus without conception, and DBCP exposure. Results are given in Table VI-2. The mean log sperm counts were negatively correlated with the current year (year of the study) durations of exposure ($p=0.018$). An exposure-related trend for low mean log sperm count was observed for men with up to 1-8 years of past exposure but was not significant. These results were confounded by the fact that many of the men with past exposures also had been exposed during the study year.

Takahashi et al. (1981) studied the frequency distribution of sperm counts among pineapple field workers and men of the general population of an area of high DBCP use on Molokai, Hawaii. At least 20 other pesticides were in use on the island, but only DBCP and ethylene dibromide (EDB) were suspected of interfering with spermatogenesis. Semen samples were obtained from 32 men, 13 of whom had been occupationally exposed to DBCP; 19 men of the Molokai general population had no known exposure to DBCP or EDB. Ten of the controls smoked marijuana with a frequency ranging from occasional use to 15 marijuana cigarettes per day. Five of the marijuana users (those who used >2 cigarettes per week) were excluded, leaving 14 men in the control group. One of the exposed men smoked 1 marijuana cigarette per week. The results of the sperm count analyses are presented in Table VI-2. Analyses of the frequency distribution were performed comparing the exposed workers with controls ($X^2=8.13$, $p<0.02$), the workers with the controls plus a group of men of the Fertile Population of Honolulu (n=72) as determined by Rogers et al. (1979) (X^2=364, $p<0.001$). Statistical analysis comparing the workers with the Fertile Population men also indicated a highly significant difference (z=15.43). No significant difference was found when workers were compared with the Infertile Population men (n=72) (Rogers et al., 1979) or when the control group was compared with the Fertile Population men. No significant difference was found with respect to semen volume, sperm motility or frequency distribution of abnormal sperm when the workers were compared with the control group or the Fertile Population group. The mean and median sperm counts of the heavy marijuana users ($\geq$2 cigarettes per week) were 17.8 and 1.5 million/mℓ, respectively, compared with 81 and 89 million/mℓ, respectively for the occasional user.

An epidemiologic study of agricultural workers of banana plantations in Israel was conducted to determine if DBCP exposure resulted in an increased incidence of spontaneous abortion (Kharrazi et al., 1980). Married male banana workers from 14 kibbutzim consisted of 102 men, 62 of whom were interviewed. The others were either unavailable or refused to participate. The number of men who had had direct contact, ranging from 1 to 20 consecutive seasons, was 56. The incidences of spontaneous abortion per pregnancy in the wives of the men before DBCP exposure and in the wives of the same men after the initial DBCP exposure were compared. The incidences of spontaneous abortion in a subgroup of wives who had conceived both before and after their husbands had been exposed were also compared. The results are presented in Table VI-2. Although the ages of the mothers were greater for the after-exposure determination, the age difference was not great enough to account for the higher incidences. Chi-square analyses indicated a significantly higher incidence of spontaneous abortion after DBCP exposure for both comparisons. None of the live births resulted in complications or abnormal children. The investigators suggested an association of spontaneous abortion with decreased sperm counts and increased frequency of abnormal sperm cells; however, semen samples were not collected from the workers.

A prospective study of occupational exposure to DBCP of Hawaiian pineapple field workers, conducted by the National Institute for Occupational Safety and Health (NIOSH), is in progress (Landrigan et al., 1983). The results of an industrial hygiene survey indicated that field workers' exposure to DBCP were generally <1 ppb (0.0097 mg/m³), but in some cases, may have been as high as 1800 ppb (17.4 mg/m³). The study population obtained from two plantations consisted of 35 field applicators, 27 maintenance workers and 29 nonexposed plantation workers. The participation rate was 80%. The decrease in mean sperm count from the beginning to the end of the growing season was 1.17 million/mℓ for the nonexposed group, 1.0 million/mℓ for the applicators and 1.1 million/mℓ for the maintenance workers. The differences were not significant. Further analyses are planned to correlate sperm count with exposures.

Special Groups at Risk

As documented by the preceding text, men can be considered a special group at risk because of the antispermatogenic effects of DBCP. Chemical production workers and agricultural workers who handle DBCP may be more susceptible to environmental exposure because of their additional exposure to the chemical in the workplace. Men who already have inherently low sperm counts, or whose sperm counts have been reduced by other factors, such as marijuana use (Takahashi et al., 1981), may be especially vulnerable to becoming azoospermic at less intense or shorter durations of exposure.

Summary

A distinct odor was detected by two human volunteers exposed to 16.4 mg/m³ DBCP in a chamber for an unspecified period of time (Torkelson et al., 1961).

Effects of DBCP exposure on spermatogenesis have been observed in workers employed at chemical production plants and in agricultural workers. Reduced sperm counts and azoospermia occurred in employees of the Occidental Chemical Company who had been involved in DBCP production (Whorton et al., 1977, 1979). The reduction in sperm count was related to the duration of the exposure. Histological examination of testicular biopsy samples indicated that the longer the periods of exposure, the more pronounced the reduction in number of spermatogenic elements in seminiferous tubules (Biava et al., 1978). Similar findings of reduced sperm count as well as increased levels of FSH were observed in employees of the Shell Oil Company at a production plant in Mobile, Alabama where exposure periods had been <1 year (Lipshultz et al., 1980). No difference in mean sperm counts was observed for employees of a Shell plant in Denver, Colorado, who had produced DBCP for 19.5 years except for the mean log differences for the >1000 hour group when analyzed by the modified t test. One reason for the discrepancy is that the production of DBCP in Denver had been terminated for a period of time sufficiently long for the exposed men to recover sperm counts. The men at the Mobile plant had been exposed to DBCP more recently. Some recovery of sperm counts in the Mobile, Alabama cohort was observed 18 months after DBCP exposure ceased (Lantz et al., 1981).

At the Dow Chemical Company plant in Midland, Michigan, employees in jobs requiring more direct and more prolonged exposure to DBCP had greater frequencies of low counts, reduced testicular size and higher FSH levels than employees in jobs requiring less intense exposures.

Azospermia and oligospermia were found with high incidence in employees of a chemical production plant in Israel (Potashnik et al., 1979). The azoospermics had the longest periods of exposure; the oligospermics had exposure periods intermediate between the azoospermics and the workers with >20 million sperm/mℓ. Recovery of sperm counts and improved sperm counts among the azospermic and the oligospermic men was found when their semen was reanalyzed 4 years later (Potashnik, 1983).

Nondisjunction of the y-chromosome was found with significantly increased frequency over background in employees of a chemical plant producing DBCP when compared with nonexposed employees (Kapp et al., 1979).

Reduced sperm counts were also found among agricultural workers (applicators, formulations, farmers, salesmen, researchers) potentially exposed to DBCP. Exposed cohorts from South Carolina, Florida, Arkansas, Alabama, Tennessee, Mississippi, California, Texas and New Jersey were studied by the Human Effects Monitoring Branch of the Office of Pesticide Programs (U.S. EPA, 1978). A "Use Index," defined as the total number of pounds of life time use ÷ total number of days of use for each individual of these cohorts, was calculated. Use indices correlated with frequency distributions among sperm count categories (i.e., the greater the index, the lower the sperm count). A significant negative exposure-related trend in mean log sperm count was also found among California agricultural workers with DBCP exposure during the year of the study (Glass et al., 1979). Men with exposures >2 months had significantly lower counts than those exposed for <2 months. Significantly reduced sperm counts were also found in pineapple workers of Molokai, Hawaii, who had known histories of DBCP and EDB exposure when compared with nonexposed men of the general population of Molokai or compared with a group of fertile men of Honolulu (Takahashi et al., 1981). Evidence that DBCP exposure of the father resulted in increased rates of spontaneous abortions in the mothers was obtained in Israel banana plantation workers (Kharrazi et al., 1980). A prospective study of Hawaiian pineapple field workers exposed to DBCP, conducted by NIOSH, is in progress (Landrigan, 1983). Preliminary results indicate no significant differences were found for the change in sperm count from preseason to postseason between exposed and nonexposed workers, but future analyses are planned.

Thus, the effects of DBCP in humans are reduced or obliterated spermatogenesis in chemical plant workers and agricultural workers depending upon the intensity and duration of DBCP exposure. Some recovery of sperm counts occurs when DBCP exposure ceases; the amount of time required depends upon the intensity and duration of the exposure.

VII. MECHANISMS OF TOXICITY

General Toxicity

Exposure of animals to DBCP has resulted in toxicity to the testis and epididymis, the renal tubules, the liver and several other organs (see General Toxicity and Target Organ Toxicity Sections in Chapter V). Chronic oral exposure has resulted in tumor induction in the forestomach, and chronic inhalation exposure has resulted in the nasal cavity tumor induction and other upper respiratory tissues (see Carcinogenicity Section in Chapter V). DBCP was mutagenic in prokaryotic and eukaryotic systems (see Mutagenicity Section in Chapter V). Human exposure to DBCP has been associated with reduced spermatogenesis and infertility (Chapter VI).

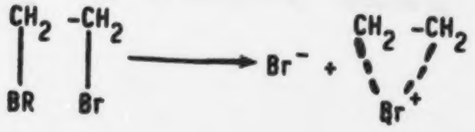
All of these toxic effects have been attributed to the action of a proposed metabolite of DBCP that is capable of binding covalently with nucleophilic sites on macromolecules such as DNA, RNA and protein. Kato et al. (1979b) demonstrated that when ^{14}C-DBCP was incubated with the S-9 fraction from rat liver homogenates, protein became labeled with radioactivity. No radioactivity was associated with protein when the S-9 fraction had been boiled before incubation or when ^{14}C-DBCP was incubated only with bovine serum albumin. The enzyme-dependent binding of radiocarbon to protein was stimulated by the addition of NADPH to the incubation system and prevented by the addition of sesamex, an inhibitor of microsomal oxidation. It was concluded, therefore, that a reactive metabolite of DBCP, formed by the action of microsomal enzymes, became covalently bound to protein *in vitro*.

The role of DBCP binding to protein in the induction of necrosis in target organs was investigated _in vivo_ in male Wistar rats that had been orally treated with doses of 20-400 mg ^{14}C-DBCP/kg bw (Kato et al., 1980). The majority of radiolabel was found in the liver and the kidney and, to a lesser extent, in the testis. The majority of the total radioactivity in the organs was bound to tissues in a form other than parent ^{14}C-DBCP. Only a small percentage of the total radioactivity was associated with parent ^{14}C-DBCP (see Distribution and Retention Section in Chapter III). The extent of binding was dose-related. The GSH content of the liver and kidney was depleted in a dose-related manner that coincided with the dose-related binding of radiocarbon to the tissues. Pretreatment of the rats with phenobarbital, an inducer of microsomal enzyme activity, before ^{14}C-DBCP administration, enhanced the binding to macromolecules and the depletion of GSH while potentiating the hepatic and renal toxicity of DBCP. Pretreatment of the rats with SKF-525A, an inhibitor of microsomal enzymes, reduced the extent of GSH content depletion and binding of radiocarbon to tissue, while having a protective effect against the hepatic and renal toxicity of DBCP. Similar results to those observed for SKF-525A were obtained when GSH was co-administered with ^{14}C-DBCP. Pretreatment of the rats with any of these metabolic modifiers had no effect on the binding of radiocarbon to the testis. The results of the _in vivo_ study support the hypothesis that a toxic metabolite of DBCP, formed by the mixed function oxidase system, binds covalently to liver and kidney macromolecules, interfering with their function, thus effecting the toxicity.

The reaction of GSH with electrophilic toxic substances and the subsequent excretion of mercapturic acid conjugates represents a mechanism of detoxication (Ketterer et al., 1983). If the concentration of toxicant is sufficiently high to substantially deplete the GSH content, then further conjugation is prevented. The toxic agent is free to exert its toxic effect, that is, react with other nucleophilic sites on tissue macromolecules.

Kluwe et al. (1981) investigated the ability of DBCP to deplete GSH content of target organs with respect to toxicity in male ICR mice (15-209) and male F344 rats (150 g). The animals were treated intraperitoneally with single injections of 0, 33 or 330 mg DBCP/kg bw. The animals were killed at 11:00 a.m. to minimize the effect of diurnal variation. In mice and rats, DBCP reduced hepatic and renal (NPS) nonprotein sulfhydryl content in a dose-related manner. The NPS content of lung, testis and stomach was also decreased, but to a lesser extent. The depletion of GSH was shown to be enzyme-dependent in an in vitro experiment. Pretreatment of the animals with 100 ppm of PBB (polybrominated biphenyls), inducers of microsomal enzymes in the diet for 18 days, or with a subcutaneous injection of 600 mg/kg piperonyl butoxide, an enzyme inhibitor, 90 minutes before DBCP administration, resulted in significantly less GSH depletion in liver and kidney than when DBCP alone was given ($p<0.05$). PBB pretreatment also protected the mice from DBCP toxicity: the acute intraperitoneal LD_{50} was raised from 175 to 230 mg/kg bw ($p<0.05$). Pretreatment with piperonyl butoxide had no effect on the LD_{50} as compared with controls. These effects were in contrast to the potentiating effect in rats of phenobarbital, another microsomal inducer, shown by Kato et al. (1980). Pretreatment of mice with a subcutaneous injection of 75 mg/kg of the inhibitor SKF-525A, 90 minutes before injection of DBCP, had no effect on GSH depletion (also in contrast to Kato et al., 1980). The action of these metabolic modifiers indicated that an enzymatic reaction was necessary to produce the reactive intermediate. Kluwe et al. (1981) explained that piperonyl butoxide inactivates cytochrome P-450. If cytochrome P-450 was inactivated, resulting in microsomal oxidation inhibition of DBCP to an electrophilic metabolite, GSH would not be depleted. If PBB induced microsomal enzymes of other hypothetical DBCP metabolic pathways, different metabolites might be formed that are not capable of reacting with GSH. In a later study, (Kluwe et al., 1982), a subcutaneous dose in corn oil of 80 mg DBCP/kg bw to male F344 rats (200-280 g) resulted in a transient decrease in GSH content of the liver and epididymis 4 and 2 hours post-treatment, respectively, but not kidney or testis. The depletion of hepatic GSH was dose-related in that no loss of GSH was observed at 10 or 20 mg/kg bw, whereas a 50% reduction in GSH was found at 80 mg/kg bw at 2 hours following treatment. GSH was not depleted in the kidney in this study as it had been in Kluwe et al. (1981). The dose of DBCP administered in the later study was ~4 times lower than the highest dose in the earlier study. Pretreatment of rats with intraperitoneal injections of diethyl maleate, a known depletor of GSH, potentiated the renal and testicular toxicity (histopathologic changes) of DBCP to a greater extent than the hepatic toxicity (Kluwe et al., 1982). As discussed in the General Toxicity and Target Organ Toxicity Sections in Chapter V, the liver is not as greatly injured by DBCP administration as the kidney or the testis in rats. While depletion of GSH in the liver coincided with the hepatotoxic effect of DBCP, the content of GSH in the kidney and testis was not depleted (Kluwe et al., 1982). The increased renal toxicity, however, of DBCP following depletion of GSH by diethyl maleate may have indicated that GSH had a protective effect. The author concluded, therefore, that the acute toxicity of DBCP to renal and testicular tissue may not result from reduced GSH content in these tissues; however, this may be the case in the liver. The results also suggested that the liver is the major site of DBCP metabolism and detoxification in line with other studies. If hepatic GSH is depleted, a threshold for the non-GSH-conjugated-metabolite to effect extrahepatic damage may be reached.

The identity of the electrophilic metabolite has been the subject of speculation and investigation. The necessity of the mixed function oxidase system for the binding of radiocarbon from ^{14}C-DBCP *in vitro* and *in vivo* (Kato et al., 1979b, 1980), the depletion of glutathione content of liver (Kato et al., 1980; Kluwe et al., 1981, 1982), and the appearance of identical mercapturic acids in the urine of rats, whether treated with DBCP, epichlorohydrin or epibromohydrin, indicated that an epoxide or epoxides are responsible for toxicity (Jones et al., 1979). In the metabolic scheme proposed by Jones et al. (1979) (see Metabolism Section in Chapter III), four epoxides capable of conjugating with GSH are formed. Kale and Baum (1982b) found that DBCP was a less potent mutagen in *Drosophila melanogaster* than was 1,2-dibromoethane. In addition, 1,2-dibromoethane and 1,2-dibromopropane were mutagenic in *D. melanogaster*, but 1,3-dibromopropane and 1,1-dibromoethane were not in the study by Vogel and Chandler (1974). Vogel and Chandler (1974) suggested that the vicinal position of the bromine atoms on 1,2-dibromoethane reacted in solution to form unstable bromonium ions that act as alkylating agents:



Kale and Baum (1982b) argued that while the substitution of the chlorine atom on C-3 of DBCP should not prevent the formation of the bromonium ion (NIOSH, 1978), the substitution may have reduced the alkylating potential. The formation of bromonium ions was thought to occur spontaneously rather than by an enzyme catalyzed reaction (Vogel and Chandler, 1974).

Jones et al. (1979) attempted to attribute the testicular and renal toxicity of DBCP in rats to different intermediates in the metabolic pathway of DBCP (see Metabolism Section in Chapter III). Jones et al. (1979) suggested that α-chlorohydrin may have been responsible for the observed infertility in man (Chapter VI), since it has caused sterility in animals and inhibited glycolysis in human sperm. Oxalic acid was also implicated since it produced spermatocoeles in the caput epididymis of rats. Oxalic acid and epichlorohydrin have both been associated with renal damage. Kluwe et al. (1983), however, conducted an extensive comparative toxicologic study of the effects of DBCP, epichlorohydrin, α-chlorohydrin and oxalic acid in rats to answer the question whether all of these compounds exert their nephrotoxic and testicular toxic effects by the same mechanism. Male F344 rats were given single subcutaneous injections of the maximum tolerated doses: 80 mg DBCP/kg bw; 75 mg epichlorohydrin/kg bw; 125 mg α-chlorohydrin/kg bw or 75 mg oxalic acid/kg bw. DBCP and epichlorohydrin were given in corn oil and α-chlorohydrin and oxalic acid were dissolved in water. Differences in effects among the various treatments compared with vehicle controls were observed. DBCP and epichlorohydrin produced polyuria; DBCP, α-chlorohydrin and oxalic acid produced glucosuria; DBCP and α-chlorohydrin produced proteinuria and increased urinary excretion of

Na^+; DBCP and oxalic acid treatments resulted in increased serum creatinine and BUN; DBCP and epichlorohydrin increased serum GPT levels. All compounds except oxalic acid resulted in hepatocellular swelling and decreased testicular weight; all compounds except α-chlorohydrin decreased the thymus weight. DBCP, epichlorohydrin and α-chlorohydrin administration resulted in depressed sperm count in cauda epididymis. Epichlorohydrin and α-chlorohydrin exposure resulted in increased percentages of abnormal sperm to 12.5% from a control value of 0.5%; DBCP and oxalic acid did not. DBCP, epichlorohydrin and α-chlorohydrin treatment all resulted in lesions of the forestomach: pustules, hyperkeratosis, erosion, ulceration and edema. The incidences and onset of histopathologic changes in the kidney, testis and epididymis for the four chemical treatments were compared. As seen in Table VII-1, early morphological changes in the kidney were observed for DBCP, epichlorohydrin and α-chlorohydrin. Later effects of DBCP were not matched by the other compounds. In the testis and epididymis, similar incidences of histological changes were observed among DBCP, epichlorohydrin and α-chlorohydrin, but not oxalic acid. The authors concluded that DBCP, epichlorohydrin and alpha-chlorohydrin had similar effects on the forestomach, testis and epididymis. These effects were not mediated by metabolism to oxalic acid. Furthermore, the nephrotoxicity of DBCP did not resemble that of any of the metabolites studied.

Mechanism of Testicular Effects

The mechanism by which DBCP exerts its antispermatogenic effects in animals and man is not known. The action of a metabolite of DBCP as an alkylating agent binding to DNA (Kato et al., 1980) of spermatogenic cells may represent the mechanism. Kale and Baum (1982b) proposed that a bromium

TABLE VII-1

The Comparative Toxicity of DBCP and Its Metabolites Epichlorohydrin, α-Chlorohydrin and Oxalic Acid in Rats Treated Subcutaneously with Maximum Tolerated Doses[a]

Number of Days Post-treatment	Lesions	Chemical Administered[b]				
		Control	DBCP	ECH	ACH	OA
				INCIDENCE[c]		
	KIDNEY					
1	Early morphological changes	0/8	4/8	5/8	3/8	0/8
	Birefringent crystals in tubules at corticomedullary junction	0/8	0/8	0/8	0/8	8/8
3	Degeneration or necrosis in outer medulla	0/8	8/8	0/8	0/8	0/8
	Birefringent crystals and focal necrosis at cortiomedullary junction	0/8	0/8	0/8	0/8	8/8
8	Necrosis and regeneration in outer medulla	0/8	8/8	0/8	0/8	0/8
	Birefringent crystals and focal regeneration at corticomedullary junction	0/8	0/8	0/8	0/8	3/8
25	Tubular dilatation, casts, regeneration in outer medulla	0/8	8/8	0/8	0/6	0/8
	Tubular necrosis in cortex, congestion, hemorrhage	0/8	0/8	0/8	2/6	0/8
75	Karyomegaly	0/8	6/8	0/8	0/8	0/8
	Lymphocytic infiltration	0/8	3/8	0/8	0/8	0/8
	Focal tubular necrosis, regeneration and tubular dilation	0/8	0/8	0/8	3/8	0/8

TABLE VII-1 (cont.)

Number of Days Post-treatment	Lesions	Chemical Administered[b]				
		Control	DBCP	ECH	ACH	OA
		INCIDENCE[c]				
	TESTES					
3	Vacuolar degeneration of the seminiferous tubular epithelium	0/8	4/8	4/8	2/8	0/8
8	Degeneration or necrosis of the seminiferous tubular epithelium, edema, aspermatogenesis, multinucleated cells	0/8	8/8	8/8	5/7	0/8
25	Degeneration or necrosis of the seminiferous tubular epithelium, edema, aspermatogenesis, multinucleated cells, tubular atrophy	0/8	7/8	5/8	5/6	0/8
75	Seminiferous tubular atrophy, edema	0/8	7/8	7/8	4/8	0/8
	EPIDIDYMIS					
1	Vacuolar degeneration of the tubular epithelium and exfoliated cells in lumen	0/8	0/8	4/8	6/8	0/8
3	Vacuolar degeneration of the tubular epithelium and exfoliated cells in lumen	0/8	6/8	0/8	0/8	0/8
	Exfoliated cells in lumen, no tubular degeneration	0/8	0/8	6/8	7/8	0/8
	Impacted sperm in tubules	0/8	0/8	0/8	3/8	0/8

TABLE VII-1 (cont.)

Number of Days Post-treatment	Lesions	Chemical Administered[b]				
		Control	DBCP	ECH	ACH	OA
		INCIDENCE[c]				
	EPIDIDYMIS					
8	Dilated tubular lumens, edema	0/8	8/8	8/8	0/7	0/8
	Exfoliated cells in lumens	0/8	4/8	4/8	4/7	0/8
	Sperm granuloma	0/8	0/8	6/8	5/7	0/8
25	Exfoliated cells in lumens	0/8	7/8	4/8	3/6	0/8
	Sperm granuloma	0/8	0/8	3/8	3/6	0/8
	Spermatocoele	0/8	0/8	2/8	0/6	0/8
75	Exfoliated cells in lumens	0/8	7/8	7/8	4/8	0/8
	Sperm granuloma	0/8	0/8	0/8	1/8	0/8

[a] Source: Kluwe et al., 1983

[b] DBCP = 1,2 dibromo-3-chloropropane; ECH = epichlorohydrin; ACH = α-chlorohydrin; OA = oxalic acid

[c] Incidences are represented as the number of rats with the lesion over the number of rats examined.

ion was responsible for DBCP-induced sex linked recessive lethal mutations in *D. melanogaster* spermatogonia. Lee and Suzuki (1981) suggested that a reactive epoxide reacted with DNA of premeiotic germ cells in prepubertal male mice, resulting in unscheduled DNA synthesis (see Mutagenicity Section in Chapter V); however, the effect was not observed in spermatogenic cells of adult mice. DBCP administration to male rats resulted in dominant lethal mutations in the early spermatid stage (Saito-Suzuki et al., 1982). The above examples that DBCP or a metabolite of DBCP can interact with germ cell DNA to cause mutuation may also explain the azoospermia, oligospermia and degeneration of seminiferous tubule epithelium observed as a result of DBCP exposure in animals (Rao et al., 1982, 1983) and man (Chapter VI).

Biava et al. (1978) analyzed by light and electron microscopy the testicular biopsy specimens from 10 volunteers in the Whorton et al. (1977) study of infertility in chemical plant workers exposed to DBCP (Table VII-2). The authors suggested that spermatogenesis was depressed at the stem-cell level, the earliest stage, by the selective action of an alkylating agent. Stem-cells are located in the outermost areas of seminiferous tubules where they might be more accessible to toxicants circulating in the blood. On the other hand, Sertoli cells, which were not affected by DBCP treatment, are important elements of the blood-testis barrier, forming an inner compartment where germ cells differentiate into mature spermatozoa. No other testicular lesions could be attributed to DBCP exposure. If the stem-cells are reduced in number in the outer portion of the tubule, fewer spermatogonia would develop for further production of sperm cells. However, the logic behind these speculations is not flawless. Toxicity to later stages of sperm development (or general toxicity to all stages) may produce

TABLE VII-2

Summary of Clinical and Morphological Data from the Sperm and Biopsies of 10 Patients[a]

Patient No.	Age (years)	Exposure to DBCP (years)	Examination of Sperm			Testicular Spermatogenic Activity[b]	Age of Last Child (years)
			Count (x10^4 cells)	Mobility	Morphology		
1	26	0.25	54	normal	normal	4+	none
2	30	1.75	vasectomy	NA	NA	3+	4
3	26	3.5 exp.[c] 3.0 no exp.	190	normal	normal	3+	wife pregnant
4	29	1	11	normal	18% abnormal	2+	5
5	53	lab. worker 1 hour/day x 7	23	reduced	normal	2+	21
6	28	3.5	vasectomy	NA	NA	1+	4
7	24	3 absent 20 months for med. reasons	1	reduced	normal	1+	3
8	35	2 absent 2, then 3	0	NA	NA	1+	none
9	37	10.5	0	NA	NA	0	11
10	39	10	0	NA	NA	0	11

[a] Source: Biava et al. (1978)

[b] Normal, 4+; slightly reduced, 3+; moderately reduced, 2+; markedly reduced, 1+; absent, 0.

[c] Exposure

NA = Not applicable

similar effects. Biava et al. (1978) found that spermatogenic activity was either absent or moderately to markedly decreased in seven subjects, and there was an apparent correlation between reduction in spermatogenesis and the duration of exposure.

Summary

A reactive metabolite of DBCP is widely thought to act as an alkylating agent capable of covalent binding with nucleophilic sites on biological macromolecules, such as DNA, RNA and protein (Kato et al., 1979b, 1980; Kluwe et al., 1981, 1982). Evidence for the reactive metabolite has been obtained from observations that radiocarbon from ^{14}C-DBCP binds to tissues of target organs in a form other than parent compound (Kato et al, 1980); that DBCP administration depletes GSH (Kato et al., 1980; Kluwe et al., 1981, 1982); that metabolic modifiers, such as phenobarbital, SKF-525A, PBB, or diethyl maleate altered the binding of radioactivity to tissue, toxicity and depletion of GSH (Kato et al., 1980; Kluwe et al., 1981); and from *in vitro* experiments demonstrating the necessity of microsomal oxidation before binding occurs (Kato et al., 1979). Among the possibilities of reactive compound are epoxides (Jones et al., 1979) and a bromium ion (Kale and Baum, 1982b).

It has also been suggested that intermediate and end metabolites of DBCP, such as epichlorohydrin (a stable epoxide), α-chlorohydrin and oxalic acid, may in part account for the wide variety of toxic effects (Jones et al., 1979). Kluwe et al. (1983) demonstrated that DBCP, epichlorohydrin and α-chlorohydrin may produce toxic effects in testis, epididymis and forestomach by similar mechanisms. The renal effects of DBCP were unlike those of oxalic acid, epichlorohydrin and α-chlorohydrin.

The testicular effects of DBCP are a major concern since these effects are observed in humans (Chapter VI). An alkylating metabolite of DBCP that is capable of interacting with DNA of spermatogenic cells may be responsible for toxicity. Positive results for recessive lethal mutations in D. melanogaster (Kale and Baum, 1980b), unscheduled DNA synthesis in premeiotic germ cells in mice (Lee and Suzuki, 1981) and dominant lethal mutations in rats (Saito-Suzuki et al., 1982) indicate that the metabolite interacts with the DNA of spermatogenic cells. On the basis of histological examination of human testicular biopsies, it was suggested that the alkylating agent suppresses spermatogenesis at the stem-cell level. Stem cells lie in the outermost areas of seminiferous tubules, unprotected by the blood-testis barrier (Biava et al., 1978).

VIII. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

The quantification of toxicological effects of a chemical consists of an assessment of the noncarcinogenic and carcinogenic effects. In the quantification of noncarcinogenic effects, an ADI is calculated. The AADI and HA values for the chemical are then calculated to define the appropriate drinking water concentrations to limit human exposure. For ingestion data, this approach is illustrated as follows:

$$\text{ADI} = \frac{\text{(NOAEL or LOAEL in mg/kg/day) (body weight in kg)}}{\text{Uncertainty/Safety Factor}} = \text{mg/day}$$

$$\text{AADI} = \frac{\text{ADI}}{\text{Drinking water volume in } \ell\text{/day}} = \text{mg/}\ell$$

where:

NOAEL = no-observed-adverse-effect level
LOAEL = lowest-observed-adverse-effect level
Body weight = 70 kg for adult or 10 kg for child
Drinking water volume = 2 ℓ/day for adults or 1 ℓ/day for children
Uncertainty/Safety Factor = 10, 100 or 1000

Utilizing these equations, the following drinking water concentrations are developed for noncarcinogenic effects:

1. A 1-day HA for 10 kg child
2. A 1-day HA for 70 kg adult
3. A 10-day HA for 10 kg child
4. A 10-day HA for 70 kg adult
5. A lifetime AADI for a 70 kg adult

The distinctions made between the HA calculations (items 1-4) are associated with the duration of anticipated exposure. Items 1 and 2 assume a single acute exposure to the chemical. Items 3 and 4 assume a limited period of exposure (possibly 1-2 weeks). The HA values will not be used in establishing a drinking water standard for the chemical. Rather, they will be used as informal scientific guidance to municipalities and other organizations when emergency spills or contamination situations occur. The AADI value (item 5) is intended to provide the scientific basis for establishing a drinking water standard based upon noncarcinogenic effects.

A NOAEL or LOAEL is determined from animal toxicity data or human effects data. For animal data, this level is divided by an uncertainty factor because there is no universally acceptable quantitative method to extrapolate from animals to humans. The possibility must be considered that humans are more sensitive to the toxic effects of chemicals than are animals. For human data, an uncertainty factor is also used to account for the heterogeneity of the human population in which persons exhibit differing sensitivities to toxic chemicals. An Office of Drinking Water (ODW) modification of the guidelines set forth by the National Academy of Sciences (NAS, 1977, 1980) are typically used in establishing uncertainty factors as follows:

- An uncertainty factor of 10 is used when good acute or chronic human exposure data are available and supported by acute or chronic toxicity data in other species.
- An uncertainty factor of 100 is used when good acute or chronic toxicity data identifying NOEL/NOAEL are available for one or more species, but human data are not available.
- An uncertainty factor of 1000 is used when limited or incomplete acute or chronic toxicity data are available or when only the acute or chronic toxicity data identify a LOAEL (but not NOEL/NOAEL) for one or more species are available.

The uncertainty factor used for a specific risk assessment is judgmental. Factors that cannot be incorporated in the NAS/ODW guidelines (NAS, 1977) for selection of an uncertainty factor, but must be considered, include the following: 1) the quality of the toxicology data, 2) the significance of the adverse effect, and 3) the existence of counterbalancing beneficial effects.

If toxicological evidence requires the chemical to be classified as a potential carcinogen, mathematical models are used to calculate the estimated excess cancer risks associated with the ingestion of the chemical by drinking water. The bioassay data used in these estimates are from animal experiments. In order to predict the risk for humans, these data must be converted to an equivalent human dose. This conversion includes correction for noncontinuous animal feeding, nonlifetime studies and for the difference in size. The factor that compensates for the size difference is the cube root of the ratio of the animal and human body weights. It is assumed that the average human body weight is 70 kg and that the average human consumes 2 ℓ of water per day. The multistage model is then fit to the equivalent human data to estimate the risk at low doses. The upper 95% confidence limit of this estimate is used. Excess cancer risks can also be estimated using other models such as the one-hit model, the Weibull model, the logit model and the probit model. There is no basis in the current understanding of the biological mechanisms involved in cancer to choose among these models. The estimates of low doses for these models can differ by several orders of magnitude.

The scientific data base used to calculate and support the setting of risk rate levels has an inherent uncertainty. This is because the tools of scientific measurement, by their very nature, involve both systematic and random error. In most cases, only studies using experimental animals have been performed. There is thus uncertainty when the data are extrapolated to humans. When developing risk rate levels, several other areas of uncertainty exist, such as 1) incomplete knowledge concerning the health effects of contaminants in drinking water, 2) the impact of test animal age, sex and species and the nature of target organ systems examined on the toxicity study results, and 3) the actual rate of exposure of internal targets in test animals or humans. Dose-response data are usually only available for high levels of exposure, not for the lower levels of exposure for which a standard is being set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects.

The National Academy of Sciences (NAS, 1977, 1982) did not calculate a drinking water standard for DBCP because they felt that contaminants of DBCP might be responsible for the observed adverse effects in humans and animals. However, the Office of Drinking Water (ODW) of the U.S. EPA has given some guidance in this area. The upper limit excess lifetime cancer risk associated with 50 ng/ℓ of DBCP is 9.0×10^{-6} assuming a consumption of 2 ℓ of contaminated water per day and an average adult body weight of 60 kg. The toxicity-based drinking water concentration is 50 ng/ℓ (Cotruvo and Melone, 1983). NIOSH (1978) has recommended that a ceiling of 10 ppb (0.1 mg/m^3) be set for occupational exposure to DBCP. In 1977, the Occupational Safety and Health Administration (OSHA) proposed to set a permissible exposure limit for DBCP at 1 ppb (0.01 mg/m^3) for an 8-hour TWA exposure and a mean ceiling of 10 ppb (0.1 mg/m^3) for any 15-minute period during the work-shift (42 FR 57266). These proposed limits were based on the view of OSHA that the lowest level of DBCP detectable by industrial sampling and analysis methods and the lowest level capable of being achieved is 1 ppb (0.01 mg/m^3). In 1979, the U.S. EPA (44 FR 65135) suspended the registration of all pesticide products containing DBCP.

Noncarcinogenic Effects

No reports of studies of accidental or intentional acute exposures of humans to DBCP were found in the available literature. The threshold for taste and smell of DBCP in water by humans was reported to be 0.01 mg/ℓ (Rakhmatullayev, 1969). The threshold for smell by inhalation was reported by the Dow Chemical Company to be 0.01-0.03 ppm (0.097-0.29 mg/m^3) in air (42 FR 48026). The discovery in 1977 that several employees of the Occidental Chemical Company, a plant that produced DBCP as well as other agricultural chemicals, were azoospermic (Whorton et al., 1977), prompted the conduction of epidemiological studies on the antispermatogenic effects of occupational exposure to DBCP. Studies of employees of the Occidental Chemical Company (Whorton et al., 1979), Shell Oil Company (Lipshultz et al., 1980), Dow Chemical Company (Egnatz et al., 1980) and a chemical production plant in Israel (Potashnik et al., 1979) report incidences of significantly ($p<0.05$) reduced sperm counts, including the conditions of oligospermia (<20 million sperm/mℓ semen) and azoospermia (0 sperm/mℓ semen). The incidence and severity of these conditions were positively related to intensity and duration of exposure. Quantification of exposure levels to approximate individual doses was not possible; however, airborne

TWA concentrations of DBCP for 8 hours of exposure were estimated to be <1 ppm (<9.7 mg/m³) in some studies (Whorton et al., 1977, 1979; Egnatz et al., 1980). Airborne DBCP was measured at 0.4 ppm (3.9 mg/m³) on 1 day early in 1977 (Whorton et al., 1977), and Egnatz et al. (1980) reported that air-monitoring at the Dow Chemical plant had been performed only in the last 2 years of the 12-year time period during which DBCP was produced. Consequently, there is considerable uncertainty in relating spermatogenic effects from occupational exposure to DBCP to estimates of actual ambient DBCP levels for the duration of the exposure of the affected workers. Individual monitoring of affected workers was not done. Also, occupational exposure to DBCP occurs primarily through inhalation, although dermal exposure may be involved. As discussed later, extrapolation of doses from inhalation routes to oral routes of exposure may not be appropriate, as indicated from the comparison of DBCP toxicity in animals exposed orally and by inhalation.

Increased incidences of oligospermia and azoospermia related to intensity and duration of exposure to DBCP have also been reported for agricultural workers (U.S. EPA, 1978; Sandifer et al., 1979; Glass et al., 1979; Takahashi et al., 1981). In some of the studies, quantification of exposure was attempted by calculating an individual "use index," defined as total number of pounds of DBCP used in the lifetime ÷ total number of days used (U.S. EPA, 1978). The "use index" was useful for comparing relative exposure of workers, but quantification of a dose was not possible. A prospective epidemiological study in pineapple plantation workers in Hawaii is in progress (Landrigan et al., 1983). An industrial hygiene survey indicated DBCP exposures to pineapple workers to be generally <1 ppb (<0.0097 mg/m³), but in exceptional cases, exposures may have been as high as 1.8

ppm (17.4 mg/m^3). No significant differences among the exposed and nonexposed groups were found for the difference in sperm counts measured before and after one growing season; further analyses are planned. The general exposure level of $\leq$0.0097 mg/m^3 at which no spermatogenic effects in pineapple workers were observed is 1000-fold lower than the $\leq$9.7 mg/m^3 exposure level estimated in chemical plants where the workers were azoospermic and oligospermic.

Evidence was found in a 4-year follow-up study of Israeli chemical plant workers that the antispermatogenic effect of DBCP was reversible, depending on the intensity and duration of exposure and the time since the last exposure (Potashnik, 1983). Other effects of DBCP exposure in humans included increased frequencies of nondisjunction of the Y-chromosome in chemical plant workers (Kapp et al., 1979) and increased incidences of spontaneous abortion in women whose husbands worked on banana plantations where DBCP was used (Kharrazai et al., 1980).

Reduced sperm counts and other testicular effects, as well as toxicity to renal tubules, liver, stomach and lungs have been observed in laboratory animals exposed to DBCP (see Chapter V). Reported values for acute oral LD_{50} varied within species: from 170-350 mg/kg bw in rats; 260-410 mg/kg bw in mice; 210-316 mg/kg bw in guinea pigs and 180-440 mg/kg bw in rabbits (Torkelson et al., 1961; Rakhmatullayev, 1971). The animal species appear to have equivalent sensitivity to DBCP exposure with respect to acute oral LD_{50}. DBCP was less toxic to rabbits when applied undiluted to the skin than when applied in propylene glycol (Torkelson et al., 1961). LC_{50}s for inhalation in rats ranged from 3557 mg/m^3 for 1 hour of exposure to 995

mg/m^3 for 8 hours (Torkelson et al., 1961). Acute high oral doses (100-628 mg/kg bw) of DBCP in rats resulted in biochemical changes, such as decreased levels of cytochrome P-450 (Moody et al., 1982a, Tofilon et al., 1980); increased protein and RNA content of liver (Moody et al., 1982b), and increased activity of the microsomal enzymes, aryl hydrocarbon hydroxylase and epoxide hydrolase (Suzuki and Lee, 1981). Other effects of high acute oral doses in rats (100-400 mg/kg bw) included reduced spermatogenesis (Reznik and Sprinchan, 1975), hepatocellular necrosis and degeneration of renal tubules (Kato et al., 1980).

Kluwe (1981a) conducted a study in male F344 rats to compare the effects of 40 mg/kg bw/day of DBCP, administered for 4 days, orally by gavage, by intraperitoneal injection or by subcutaneous injection. For all three routes of exposure, body weight was decreased at day 3 and rats had watery stools. BUN levels were significantly increased above control ($p<0.05$) for all routes of exposure. BUN levels after oral or subcutaneous exposures were also significantly increased above that following intraperitoneal injection ($p<0.05$), and these levels were higher ($p<0.05$) after subcutaneous injection compared with oral treatment. The specific gravity of urine was significantly decreased from the control value for all exposures ($p<0.05$) and was significantly lower after the subcutaneous route than after the intraperitoneal route ($p<0.05$). Following oral administration, the kidney-to-brain weight ratio was significantly higher than that following intraperitoneal dosing or in controls ($p<0.05$) and significantly lower ($p<0.05$) than that after subcutaneous injection. Histopathologic lesions included necrosis of proximal renal tubule cells, hepatocellular swelling, disruption of seminiferous tubule structure in the testis and degeneration of epithelial cells of the caput epididymis. The severity of lesions in liver and testis was similar following all three routes of exposure. The lesions of the kidney were qualitatively similar for all routes but were most severe following subcutaneous injection. Thus, DBCP was slightly more toxic to the kidney of rats following subcutaneous administration than following oral exposure.

Since Kluwe (1981a) was most interested in the renal effects of DBCP, he studied the dose-response for single exposure in rats using the subcutaneous route. At subcutaneous doses of DBCP of 80 and 120 mg/kg bw, effects included reduced sperm count, degeneration of the caput epididymis, loss of body weight, disheveled fur, hypothermia, diarrhea, elevated BUN and serum creatinine content, increased urinary excretion of glucose, increased levels of serum GPT, sorbitol dehydrogenase, LDH and alkaline phosphatase. At ≥40 mg/kg bw, there was increased urinary excretion of protein, GOT, LDH, N-acetyl-β-D-glucosaminase, alkaline phosphatase, γ-glutamyl-transpeptidase and Na^+ and K^+, cytoplasmic vacuolization of proximal renal tubule epithelium and hepatocellular swelling progressing to necrosis. At ≤40 mg/kg bw subcutaneously, there was no effect on body weight or clinical signs of toxicity. At 20 mg/kg bw, no adverse effects of DBCP administration were observed. Thus, 20 mg DBCP/kg bw represents a NOAEL for acute subcutaneous administration. Since similar qualitative effects were observed with subcutaneous and oral dosing with 40 mg/kg bw/day for 4 days, the 20 mg/kg bw acute subcutaneous dose may also represent a NOAEL for oral administration. The oral NOAEL may actually be <20 mg/kg bw/day, since the effects of single subcutaneous injections were more severe than those of a single oral dose.

A dose of 12.5 mg/kg bw/day of DBCP administered by gavage to pregnant Wistar rats on days 6-15 of gestation resulted in no fetotoxic or maternal toxic effects (Ruddick and Newsome, 1979). At 25 and 50 mg/kg bw/day, dams had reduced mean body weight and at 50 mg/kg bw/day, fetal body weight was reduced. Thus, 25 mg/kg bw was the LOAEL for acute oral DBCP exposure in pregnant rats.

Continuous inhalation exposure of 29 mg/m³ in rats for 14 days resulted in decreased numbers of germ cells and atrophy of a few seminiferous tubules in 1 of 2 rats. At 9.7 mg/m³, no testicular effects were observed (Saegusa et al., 1982). Therefore, 9.7 mg/m³ DBCP administered continuously for 14 days was a NOAEL for testicular effects by inhalation exposure. This concentration in air corresponds to a daily oral dose of ~3.1 mg/kg bw/day, calculated from the following expresssion:

$$\text{dose} = (9.7\ \text{mg/m}^3 \times 0.223\ \text{m}^3\text{/day} \times 0.5) \div 0.35\ \text{kg}$$

where

0.223 m³/day = the rat breathing volume in 24 hours

0.5 = assumed absorption coefficient commonly used in lieu of chemical specific data

0.35 = average bw of a rat

Subchronic dietary administration of DBCP for 90 days to groups of 14 male and 14 female Long-Evans rats resulted in dose-related effects (Torkelson et al., 1961). At ≤150 mg/kg diet there were no clinical signs of toxicity. At 450 mg/kg diet, rats had ruffled fur. At 1350 mg/kg diet, listlessness, muscular weakness and increased mortality were observed. Body weight gain was significantly depressed at ≥150 mg/kg diet for female rats and ≥450 mg/kg diet for male rats. At ≥20 mg/kg diet, female rats had significantly increased kidney-to-body weight ratios. This effect was observed in male rats only at 1350 mg/kg diet. Thus, 20 mg/kg diet was the lowest adverse effect level. The next lowest dietary level was 5 mg/kg diet, at which no adverse effects were observed. Assuming that a young growing rat in a subchronic study consumes an amount of food equal to 10% of its body weight per day (Mitruka et al., 1976), 5 mg/kg diet x 0.10 = 0.5 mg/kg bw/day. Testicular effects were not observed in the subchronic dietary study (Torkelson et al., 1961), but at a gavage dose of 70 mg/kg bw/day for 45 days or for 2.5 months testicular necrosis was observed (Rakhmatullayev, 1969, 1971). Histopathologic damage also included hepatocellular necrosis, bile duct proliferation, necrosis in spleen and cytomegaly in the nephron epithelium (Rakhmatullayev, 1969, 1971; Faydysh et al., 1970). Reduced sperm counts were observed in rats receiving multiple doses (not otherwise specified) of 10 mg/kg bw for 4-5 months (Reznik and Sprinchan, 1975). Exposure of male Sprague-Dawley rats to DBCP in drinking water for 64 days at 200, 100, 50 or 5 ppm had no significant effect on the reproductive system or liver or kidney function. However, an additional experiment with rats given DBCP in drinking water for 5 days resulted in signs of kidney toxicity (increased urinry protein and glucose levels) with the 200 and 100 ppm doses, but animals given the 50 and 5 ppm doses were not similarly evaluated for these signs of kidney toxicity (Heindel et al., 1983). Rats were not evaluated for urinary glucose and protein levels in a drinking water study with DBCP by Johnston et al. (1981).

For subchronic inhalation exposure, a no-effect level in rabbits and rats was 0.97 mg/m³, 6 hours/day, 5 days/week for 14 weeks (Rao et al., 1982, 1983). At this exposure level, there was no effect on mortality, clinical chemistry, hematology, body weight, organ weights, histopathology or testicular function. At 9.7 mg/m³ (same schedule) there was focal necrosis and multifocal atrophy of the testis, loss of spermatogenic elements and reduced sperm counts. At 97 mg/m³ these effects were more severe and other effects included reduced body weight, reduced kidney-to-body weight ratio and nodular hyperplasia of the adrenal cortex. At inhalation exposure of ≥9.7 mg/m³, 6 hours/day, 5 days/week for 13 weeks, dose-related increased incidences of nasal cavity and upper airway lesions in mice and rats, and renal tubule nephrosis, hepatic lesions and adrenal cortex lesions in rats were observed (NTP, 1982a). Thus 0.97 mg/m³, 6 hours/day, 5 days/week for 14 weeks in rabbits and rats (Rao et al., 1982, 1983) represents a NOAEL for subchronic inhalation exposure. This exposure level would correspond to an oral dose in rats of ~0.06 mg/kg bw/day and in rabbits of ~0.026 mg/kg bw/day, calculated from the following expression:

$$\text{dose} = 0.97\ \text{mg/m}^3 \times (6\ \text{hours} \div 24\ \text{hours}) \times (5\ \text{days} \div 7\ \text{days}) \times 0.5 \times I \div \text{bw}$$

where

0.5 = assumed absorption coefficient as before

I = the rat (0.223 m³/day) or the rabbit (1.11 m³/day) breathing volume in a 24-hour day (Altman and Dittmer, 1974)

bw = 0.35 kg for rats or 3.7 kg for rabbits

These oral doses estimated from inhalation NOAELs are considerably lower than actual oral doses that produce similar effects. In the Torkelson et al. (1961) subchronic dietary study, testicular effects were not observed at any dose; however, Torkelson et al. (1961) did find focal changes in testis epithelium at 48 mg/m³, 7 hours/day, 5 days/week in rats (3.2 mg/kg bw/day). Testicular effects were found only at a relatively high daily gavage dose of 70 mg/kg bw/day for 45 days to 2.5 months (Rakhmatullayev, 1969). Effects on gonadotropic function of rats were observed at oral doses of 0.5 and 5 mg/kg bw/day for 8 months (Rakhmatullayev, 1971). The NOAELs for short-term inhalation exposure in rabbits and rats, when converted to equivalent oral doses, are ~8-10 times lower than the dietary subchronic NOAEL of 0.5 mg/kg bw/day, at which no testicular, renal or hepatic effects were observed. Comparison between effects of oral and inhalation exposure indicate that the extrapolation from inhalation routes to oral routes of exposure are not appropriate for DBCP. Furthermore, oral studies take precedence over inhalation studies for evaluating the risk of exposure to DBCP in drinking water. Using the 0.4 ppm (3.9 mg/m³) exposure level in chemical production plant workers (Whorton et al., 1977) for which subchronic exposure has resulted in oligospermia and azoospermia, an equivalent oral dose of 0.20 mg/kg bw/day can be approximated by the following calculation:

$$\text{dose} = 3.9 \text{ mg/m}^3 \times 10 \text{ m}^3\text{/day} \times (5 \text{ days} \div 7 \text{ days}) \times 0.5 \div 70 \text{ kg}$$

where

10 m³/day = the human breathing volume in an 8-hour workday

0.5 = inhalation absorption coefficient

70 kg = human body weight

This approximate human equivalent oral dose is lower than the subchronic oral animal NOAEL of 0.5 mg/kg bw/day. For reasons discussed previously, it is not appropriate to permit the estimated human exposure levels to affect the risk assessment. To reiterate, individual monitoring of affected workers was not performed; although primary exposure is by inhalation, no estimates of dermal exposure were made; and extrapolation from inhalation to the oral route of exposure is inappropriate, as evidenced from comparison of toxicity in animals given DBCP orally and by inhalation.

Toxicity data from chronic oral gavage exposures of animals to DBCP are limited to studies designed to assess the carcinogenicity of the pesticide (NCI, 1977; Hazelton Laboratories, 1977, 1978). Osborne-Mendel rats treated by gavage with TWA doses of 15 and 29 mg/kg bw/day, 5 days/week (corrected TWA doses of 10.7 and 20.7 mg/kg bw/day, see Table V-5) for 64-78 weeks had dose-related decreased mean body weights and high early mortality (believed to be tumor-related). Toxic tubular nephropathy was observed in 98-100% of the treated rats. In $B6C3F_1$ mice treated by gavage with TWA doses of 110-219 mg/kg bw/day, 5 days/week (corrected TWA doses of DBCP of 78.6-156 mg/kg bw/day, see Table V-6) for 47-60 weeks, high rates of early mortality and dose-related increased incidences of toxic tubular nephropathy were observed (NCI, 1977).

In the Hazelton Laboratories (1977) study, the incidences of carcinomas of the renal tubules, stomach and liver were significantly higher ($p<0.05$) than control in Charles River rats treated with DBCP in the diet at a nominal dose of 3.0 mg/kg bw/day (2.0 mg/kg bw/day as estimated by the CAG to account for volatilization; 44 FR 65135), but not at 1.0 mg/kg bw/day

(0.68 mg/kg bw/day, as adjusted by the CAG). At the 1-year interim kill, there was no carcinogenic response in evidence; the only observed toxic effects were increased severity of interstitial nephritis in high-dose females and significantly increased ($p<0.05$) mean absolute weight of the kidney of high-dose males. The 1.0 mg/kg bw/day dose (0.68 mg/kg bw/day) for 1 year cannot be considered a no-effect level for rats: in the Hazelton Laboratories (1978) study, a dose-related increased incidence in clusters of nodules in the nonglandular mucosa of the stomach was observed in HaM/IcR mice treated with dietary doses of DBCP of 0.3, 1.0 and 3.0 mg/kg bw/day (0.28, 0.91 and 2.7 mg/kg bw/day, as adjusted by the CAG). The incidence of the stomach nodules was 13/26 in the low-dose males and 10/44 in the low-dose females compared with 0/28 and 1/32 for male and female controls, respectively. Since carcinomas and papillomas of the forestomach were also seen in the high-dose groups, the stomach nodules may represent precancerous lesions. Therefore, a NOAEL for chronic oral exposure to DBCP cannot be defined in these studies.

The 8-month gavage study in male rats (Rakhmatullayev, 1971) is inappropriate for consideration in the risk assessment. In comparison with the chronic feeding studies, the report lacks sufficient detail for a solid analysis of the data; small groups of animals (7/dose) were used; dosing was by gavage; and in comparing similar endpoints between rats sacrificed at 12 months in the chronic feeding study (Hazelton Laboratories, 1977) with rats evaluated at the end of 8 months (Rakhmatullayev, 1971), such endpoints as white blood cell count, blood cholinesterase and BUN were reported to be affected by DBCP doses as low as 0.5 mg/kg bw/day in the 8-month study, but were not affected by doses as high as 3.0 mg/kg bw/day (2.0 mg/kg bw/day as adjusted by the CAG) by 12 months in the chronic feeding study.

In the 90-day feeding study in rats by Torkelson et al. (1961), 0.5 mg/kg bw/day was a NOAEL for subchronic exposure to DBCP. A NOAEL of 0.5 mg/kg bw/day cannot be used as a NOAEL for chronic oral exposure in view of the high incidences of stomach nodules in male (50%) and female (23%) mice at dietary doses of DBCP as low as 0.3 mg/kg bw/day (0.28 mg/kg bw/day as adjusted by the CAG) in the 78-week study (Hazelton Laboratories, 1978).

Quantification of Noncarcinogenic Effects

As reviewed by Kluwe (1981a), subcutaneous injections of 40 mg DBCP/kg bw/day in adult rats for 4 days resulted in approximately equivalent toxic effects as did oral doses of 40 mg/kg bw/day for 4 days. When the dose-response of single subcutaneous injections of DBCP in adult rats was defined, 40 mg/kg bw/day resulted in cytoplasmic vacuolization of renal tubule epithelium and impaired renal function, as evidenced by increased urinary excretion of proteins and ions. At 20 mg/kg bw subcutaneously, no toxic effects were observed and this dose was considered to be a NOAEL. The data by Kluwe (1981a) indicate that the subcutaneous injection NOAEL of 20 mg/kg bw could be equivalent to an acute oral NOAEL of 20 mg/kg in adult rats. However, Kluwe et al. (1985) showed subcutaneous injection of 20 mg DBCP/kg bw to be a LOAEL for body weight and gonadal effects in 6-day-old rats. Therefore, 1-day HAs are calculated using a LOAEL of 20 mg/kg as follows:

For an adult: $$\text{1-day HA} = (20 \text{ mg/kg bw/day} \times 70 \text{ kg}) \div (1000 \times 2 \text{ ℓ/day}) = 0.7 \text{ mg/ℓ}$$

This HA is equivalent to 1.4 mg/day or 0.02 mg/kg bw/day.

For a child: $$\text{1-day HA} = (20 \text{ mg/kg bw/day} \times 10 \text{ kg}) \div (1000 \times 1 \text{ ℓ/day}) = 0.2 \text{ mg/ℓ}$$

This HA is equivalent to 0.2 mg/day or 0.02 mg/kg bw/day.

where

70 kg = weight of protected individual if an adult

10 kg = weight of protected individual if a child

2 ℓ/day = volume of water imbibed/day by a 70 kg adult

1 ℓ/day = volume of water imbibed/day by a 10 kg child

1000 = uncertainty factor appropriate for use with an animal LOAEL

The 1-day HA is substantially higher than the 0.01 mg/ℓ threshold for smell and taste in water (Rakhmatullayev, 1969).

The 90-day study by Torkelson et al. (1961) is selected for the 10-day HA calculation since the dietary administration used more closely approximates drinking water exposure than other possible studies where other methods of dosing were used and a wide dose range showing effect and no-effect levels was evaluated. The recent and as yet unpublished study by Heindel et al. (1983) where male rats were given DBCP in drinking water for 64 days is not used because female rats were not used in the study and the complete dose response for kidney toxicity was not measured to provide a NOEL. In Torkelson et al. (1961), a dietary NOAEL of 5 mg/kg diet in rats was determined. At the next highest dietary level (20 mg/kg diet), female rats had significantly increased kidney-to-body weight ratios. The NOAEL of 5 mg/kg bw/day can be used to calculate 10-day HAs as follows:

Transformed dose for rats:

$$\begin{aligned} d &= 5 \text{ mg/kg diet} \times 0.10 \text{ kg/day} \\ &= 0.50 \text{ mg/kg bw/day} \end{aligned}$$

For an adult:
$$\begin{aligned}10\text{-day HA} &= (0.50\text{ mg/kg bw/day} \times 70\text{ kg}) \div (100 \times 2\ \ell\text{/day})\\ &\sim 0.18\text{ mg/}\ell\end{aligned}$$

This HA is equivalent to 0.35 mg/day or 0.0050 mg/kg bw/day.

For a child:
$$\begin{aligned}10\text{-day HA} &= (0.50\text{ mg/kg bw/day} \times 10\text{ kg}) \div (100 \times 1\ \ell\text{/day})\\ &= 0.050\text{ mg/}\ell\end{aligned}$$

This HA is equivalent to 0.050 mg/day or 0.0050 mg/kg bw/day.

where

5 mg/kg diet = dietary level of DBCP
0.10 = assumed proportion of body weight ingested by a young growing rat/day (Mitruka et al., 1976)
70 kg = weight of protected individual if an adult
10 kg = weight of protected individual if a child
2 ℓ/day = volume of water imbibed/day by an adult
1 ℓ/day = volume of water imbibed/day by a child
100 = uncertainty factor appropriate for use with an animal NOAEL

If the permissible exposure level for an 8-hour TWA of 0.01 mg/m^3 for occupational exposure proposed by OSHA in 1977 (42 FR 57266) were converted to an equivalent oral dose, the resulting dose is 10 times smaller than the 10-day HA, calculated previously. The calculation is as follows:

$$\begin{aligned}d\text{ (mg/kg/day)} &= 0.01\text{ mg/m}^3 \times 10\text{ m}^3\text{/day} \times 5/7 \times 50\% \div 70\text{ kg bw} \times 100\%\\ &= 0.0005\text{ mg/kg bw/day}\end{aligned}$$

where

0.01 mg/m^3 = 8-hour TWA permissible exposure level
10 m^3/day = assumed human breathing volume in an 8-hour workday
5/7 = ratio of workdays to days in a week
50% = assumed inhalation absorption efficiency
100% = assumed oral absorption efficiency
70 kg = human body weight

An HA can be estimated from 0.4 ppm (3.9 mg/m³), the exposure level in chemical production workers (Whorton et al., 1977) for which subchronic exposure resulted in reduced sperm counts, as follows:

Transformed dose

$$d = 3.9\ \text{mg/m}^3 \times 10\ \text{m}^3\text{/day} \times 5/7 \times 50\% \div 70\ \text{kg bw} \times 100\%$$
$$\sim 0.20\ \text{mg/kg bw/day}$$

where the same assumptions as used in the previous calculation apply

and

$$\text{10-day HA} = (0.20\ \text{mg/kg bw/day} \times 70\ \text{kg bw}) \div (100 \times 2\ \ell\text{/day})$$
$$= 0.070\ \text{mg/}\ell$$

where, the same assumptions as used in calculating a 10-day HA for an adult from an animal NOAEL were used, with the exception of an uncertainty factor of 100, which is appropriate for a human LOAEL.

The value of 0.07 mg/ℓ is very close to the HA calculated from animal oral data; however, for reasons discussed in the Noncarcinogenic Effects Section, the HA calculated from an oral NOAEL is more appropriate than an HA calculated from an inhalation LOAEL.

A lifetime AADI is not calculated because forestomach nodules were found with the lowest dietary feeding level of DBCP in a chronic study with mice. This dose level of DBCP was as low as or lower than those in subchronic or chronic studies in rats also considered for calculation of an AADI.

A summary of the data used to calculate the recommended 1-day and 10-day HAs for DBCP is provided in Table VIII-1.

TABLE VIII-1

Summary of Data Used to Derive HAs and AADI for DBCP

Criteria	Animal Dose	Duration	Effect	Value for HA or AADI		Reference
				Child	Adult	
1-Day HA	20 mg/kg bw	single dose	LOAEL for testicular and clinical effects	0.2 mg/ℓ (0.2 mg/day or 0.02 mg/kg bw/day)	0.7 mg/ℓ (1.4 mg/day or 0.02 mg/kg bw/day)	Kluwe, 1981a
10-Day HA	0.50 mg/kg bw/day	90 days	NOAEL for hepatic, renal, testicular and clinical effects	0.050 mg/ℓ (0.050 mg/day or 0.0050 mg/kg bw/day)	0.18 mg/ℓ (0.35 mg/day or 0.0050 mg/kg bw/day)	Torkelson et al., 1961

Carcinogenic Effects

The carcinogenicity of DBCP has been studied in rats and mice by oral (NCI, 1977; U.S. EPA, 1979a; 44 FR 65135) and inhalation routes (NTP, 1982a) and in mice by dermal application (Van Duuren et al., 1979). In the NTP (1982a) bioassay, there was dose-related significantly increased incidences of nasal cavity tumors in male and female F344 rats and $B6C3F_1$ mice that had received chronic inhalation exposures to DBCP at 5.8 and 29 mg/m^3, 6 hours/day, 5 days/week. The mice had increased incidences of pulmonary tumors as well. DBCP was positive when tested as a tumor initiator on the skin of HA:ICR Swiss mice (Van Duuren et al., 1979). Following 60 weeks of treatment with the tumor promotor, phorbol myristate acetate, a significantly increased incidence of papillomas was observed. DBCP was negative when tested as a whole carcinogen on skin; however, the incidences of distant tumors, that is, lung papillomas and papillomas and squamous cell carcinomas of the forestomach, were significantly ($p<0.05$) increased compared with controls.

In the NCI (1977) bioassay of DBCP, Osborne-Mendel rats received TWA doses of 15 and 29 mg/kg bw/day, 5 days/week by gavage (corrected TWA gavage doses of 10.7 and 20.7 mg/kg bw/day). The incidences of squamous cell carcinomas of the forestomach were 0/20 untreated control males, 0/20 vehicle treated control males, 47/50 low-dose males, 47/50 high-dose males, 0/20 untreated control females, 0/20 vehicle females, 38/50 low-dose females and 29/49 high-dose females. The incidences were significantly dose-related ($p<0.001$) and significantly increased above controls for all treated groups ($p<0.001$). The incidences of adenocarcinoma of the mammary gland in female rats were 2/20 untreated control, 0/20 vehicle control, 24/50 low-dose females and 31/50 high-dose females. The incidence in treated female rats was significantly increased over controls ($p<0.003$) and significantly dose-related ($p<0.001$).

In a chronic dietary bioassay conducted by Hazelton Laboratories (1977) for Dow Chemical Company, Charles River rats were maintained on diets containing DBCP. It was estimated that the rats had ingested 0.20, 0.68 and 2.0 mg/kg bw/day (U.S. EPA, 1979a; 44 FR 65135). The incidences of treatment-related tumors are presented in Table VIII-2.

In the NCI (1977) bioassay of DBCP in $B6C3F_1$ mice, the corrected TWA gavage doses were 81.4 and 156.4 mg/kg bw/day for males and 78.6 and 149.3 mg/kg bw/day for females. The incidences of squamous cell carcinomas of the forestomach were 0/20 untreated male controls, 0/20 vehicle male controls, 43/46 low-dose males, 47/49 high-dose males, 0/20 untreated females, 0/20 vehicle treated females, 50/50 low-dose females and 47/48 high-dose females. The dose-related trends were significant ($p<0.001$) as were the increases above controls ($p<0.001$) for all treated groups.

In the Hazelton Laboratories (1978) dietary study in HAM/ICR Swiss mice, the estimated doses were 0.28, 0.91 and 2.7 mg/kg bw/day (U.S. EPA, 1979a). Results only for the control and the high-dose groups (2.7 mg/kg bw/day) were reported. The incidences of squamous cell papillomas of the forestomach were 0/50 male controls, 6/49 high-dose males, 0/50 female controls, 6/50 high-dose females. The incidences of squamous cell carcinomas of the forestomach were 0/50 male controls, 26/49 high-dose males, 0/50 female controls and 25/50 high-dose females.

TABLE VIII-2

Number of Rats with Tumors of the Kidney, Stomach and Liver[a]

Dose (mg/kg/day)	Males				Females			
	0	0.3	1.0	3.0	0	0.3	1.0	3.0
Rats examined	48	46	46	41	48	45	47	43
Renal Tubules:								
Number with adenoma	0	0	1	6[b]	0	0	0	7[b]
Number with carcinoma	0	1	3	9[b]	0	1	0	5[b]
Total with tumors	0	1	4	15[b]	0	1	0	12[b]
Stomach Squamous Cell:								
Papilloma	0	0	0	1	0	0	0	2
Carcinoma	0	0	3	20[b]	0	0	0	8[b]
Total	0	0	3	21[b]	0	0	0	10[b]
Liver:								
Neoplastic nodules	0	4	2	3	0	2	2	3
Hepatocellular carcinoma	0	1	2	5[b]	0	1	3	0
Total	0	5[b]	4	8[b]	0	3	5[b]	3

[a] Source: U.S. EPA, 1979a

[b] Significantly higher than controls, $p<0.05$ (Fisher exact test)

DBCP was mutagenic in *Salmonella typhimurium* strains TA1535, TA1530, TA100 and TA98 and in *Escherichia coli*, with and without metabolic activation (Prival et al., 1977; Rosenkranz, 1975; Stolzenberg and Hine, 1979; Moriya et al., 1983). Some of the mutagenic potential of DBCP was attributed to epichlorohydrin (Biles et al., 1978), a contaminant as well as a metabolite of DBCP; however, a positive response was obtained from pure DBCP with S-9. DBCP was negative for mutagenicity in *S. typhimurium* strains TA1537 and TA1538 (Rosenkranz, 1975; Moriya et al., 1983). In eukaryotic systems, DBCP was positive in recessive lethal assays, in genetic crossing over assays and for chromosome breakage in *Drosophila melanogaster* (Inoue et al., 1982; Zimmering, 1983; Kale and Baum, 1982b); in dominant lethal assays in rats (Teramoto et al., 1980; Saito-Suzuki et al., 1982; Rao et al., 1983); for SCE in Chinese hamster cells (Tezuka et al., 1980); for chromosome aberrations in rats *in vivo* (Kapp, 1979) and for unscheduled DNA synthesis in germ cells of prepubertal mice (Lee and Suzuki, 1979).

Quantification of Carcinogenic Effects

In 1979, the Carcinogen Assessment Group (CAG) of the U.S. EPA evaluated the carcinogenicity data discussed above and obtained a carcinogenic potency factor for humans of 5.13 $(mg/kg/day)^{-1}$ for exposure to DBCP by ingestion. This potency factor (b_H) was generated from the tumor incidences for liver, kidney and stomach in high-dose rats from the Hazelton Laboratories study using the one-hit model for carcinogenicity (U.S. EPA, 1979b).

Using the expression: $\text{dose} = 10^{-x} \div b_H$, the corresponding doses associated with increased lifetime risks of cancer of 10^{-4}, 10^{-5} and 10^{-6} are 0.0195, 0.00195 and 0.000195 μg/kg/day, respectively, or 1.365, 0.1365 and 0.01365 μg/day, respectively, for a 70 kg human or 0.6825, 0.06825 and 0.006825 μg/ℓ, assuming an adult consumes 2 ℓ of water/day.

CAG has re-evaluated the data using the linearized multistage model developed by Crump and adopted by U.S. EPA (45 FR 79318) to generate a carcinogenic potency factor (q_1*) of 1.40 (mg/kg/day)$^{-1}$. Using the q_1* of 1.4 (mg/kg/day)$^{-1}$, the CAG (1984) has calculated a 1x10^{-6} upper limit of risk from lifetime exposure to 0.025 μg DBCP/ℓ water, assuming a daily water consumption of 2 ℓ/day and an average adult body weight of 60 kg. Lifetime exposures to 0.25 and 2.5 μg/ℓ would correspond to 10^{-5} and 10^{-4} upper limits of risk, respectively, by the CAG method.

The Shell Oil Company (1983) has derived risk assessments based on the same data base used for the CAG risk assessment, i.e., the tumor incidence for liver, kidney and stomach in rats from the Hazelton study. Using the geometric mean of five models (probit, logit, Weibull, gamma multi-hit and multistage) and three dose scaling factors (mg/kg, ppm and mg/m^3) for each data set, the Shell Oil Company calculated risks from lifetime and partial lifetime exposures to DBCP. The conclusion in the Shell Oil Company risk assessment is that partial lifetime exposures to levels of DBCP <100 ppb in drinking water poses no significant risk of an adverse health effect to humans. The risk estimates for 100 ppb exposures by the Shell methodology are found in Table VIII-3.

However, a preliminary review by the Carcinogen Assessment Group of the unpublished Shell analysis has revealed methodological inconsistencies with the Agency's practice in using cancer risk models. For example, taking the

TABLE VIII-3

Risk Estimates by Shell Methodology*

Duration of Exposure by Humans to 100 ppm DBCP	Geometric Mean of Lifetime Extra Risk Estimates with (Range)	Data Base
70 years (lifetime)	4.6×10^{-6} (1.8×10^{-13} to 2.1×10^{-3})	male rats
20 years	1.5×10^{-7} ($<1\times10^{-16}$ to 5.9×10^{-4})	male rats
10 years	3.7×10^{-8} ($<1\times10^{-16}$ to 3×10^{-4})	male rats
70 years	2.6×10^{-14}	female rats
20 years	1.7×10^{-14}	female rats
10 years	1.5×10^{-14}	female rats

*Source: Shell Oil Company, 1983

geometric mean of a series of estimates obtained from different models, as done in the analysis, has no discernable logical basis. It is clear that such observations are not log-normal distributed. Thus, the usual rationale for obtaining a combined geometric mean estimate under one model where the different estimates may have resulted from multiplicative random errors does not hold. If a threshold model was included in a group of models and the geometric mean taken, the resulting combined estimate would be zero, regardless of the risk predicted by the other models.

In a similar manner a single low estimate obtained by a probit model (i.e., a model that has no relevance for a carcinogenic response) can produce combined estimates of low risk even though more conventional approaches would give far greater risks. A combined risk obtained in this manner, that is below 1×10^{-5} for lifetime exposure, is the basis for the report's contention that no adverse health effect to humans would be expected below 100 ppb in drinking water. Notably, risks greater than 1×10^{-5} are predicted by one of the models referenced in the report.

Given the above inconsistencies, the risk derivation portion of the rationale for the conclusion that no appreciable risk exists for exposures below 100 ppb in drinking water by Shell Oil is unconvincing.

Special Considerations

High Risk Subpopulations. High risk subpopulations have not been identified in the available literature. Men are at risk because of the antispermatogenic effects of DBCP. Chemical production workers and agricultural workers who handle DBCP are more susceptible to environmental exposure because of their additional exposure to the chemical in the workplace. Men with inherently low sperm counts, or whose sperm counts have been reduced by other factors such as heavy marijuana use (Takahashi et al., 1981), may be more vulnerable and develop azoospermia with shorter durations or less intense exposures.

Multiple Pollutant Exposures. The DBCP used in the carcinogenicity studies in rodents were contaminated with low levels of such substances as epichlorohydrin, dibromoethane (NTP, 1982a) and allyl chloride (Hazelton Laboratories, 1977; 44 FR 65135). As discussed by NTP (1982a), epichlorohydrin induced nasal cavity tumors in rats at an inhalation concentration of 970 mg/m^3 (Laskin et al., 1980), and dibromoethane induced nasal cavity tumors in rats and mice at inhalation concentrations of 97 and 388 mg/m^3 (NTP, 1982b). It was estimated that the maximum level of epichlorohydrin to which animals had been exposed in the DBCP bioassays was 0.2 mg/m^3. The NTP (1982a) report concluded that while it was unlikely that the contaminants resulted in the effects observed in the DBCP bioassays, a cocarcinogenic effect could not be ruled out.

Dr. Roy Albert presented testimony to the U.S. EPA during the hearings of the Notice of Intent to Suspend Registration of DBCP (U.S. EPA, 1979b; 44 FR 65135) in which he assessed the issue of possible effects of contaminants of DBCP in the bioassays. Dr. Albert's conclusion was that the carcinogenic effect of DBCP in rats could not be attributed to contamination by allyl chloride in the NCI gavage studies performed by Hazelton Laboratories (NCI, 1977) and in the feeding study performed for Dow Chemical Company by the Hazelton Laboratories (1977). Dr. Albert did not rule out the possibility of a synergistic effect of allyl chloride. Dr. Van Duuren also testified at the hearings (44 FR 65135) that the tumorigenic response of DBCP when tested on mouse skin (Van Duuren et al., 1979) was not likely to have been the result of contaminants (allyl chloride, 0.06%, and epichlorohydrin, 0.06%).

As reviewed in Chapter VI, the chemical company employees and agricultural workers who were exposed to DBCP had also been exposed to a wide variety of other agricultural chemicals. For example, in the study of employees of the Occidental Chemical Plant (Whorton et al., 1979), production records indicated heavy use of epichlorohydrin, ethylene dibromide (EDB), carbaryl, diazinon, dinoseb, endosulfan, malathion, maneb, methyl parathion, parathion, toxaphene and zineb. For pineapple plantation workers on Molokai in Hawaii, Takahashi et al. (1981) reported aldicarb, EDB, benomyl, captafol, diazinon, ethephon, ethylene, ametryn, atrazine, bromacil, dalapon, diuron, diazinon, heptachlor, malathion, methomyl, methyl parathion, oxamyl and phenamiphos to be among the pesticides used on the island of Molokai. Takahashi et al. (1981) concluded that only EDB was suspected of having an effect on spermatogenesis. However, epichlorohydrin also has adverse effects on spermatogenesis (U.S. EPA, 1985).

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